TITLE OF PROJECT: Determination of personal exposures to Environmental Tobacco Smoke in British non-smokers.

INVESTIGATOR(S) K Phillips, J Freeman and T H Houseman

INSTITUTION: Hazleton UK

2023703470

RESEARCH ABSTRACT

Title of Project: Determination of personal exposures to Environmental Tobacco Smoke in British non-smokers.

Investigator(s): K Phillips, J Freeman and T H Houseman

Institution: Hazleton UK

ABSTRACT: In the space below, please provide a descriptive summary of your proposed research project.

We propose to investigate typical personal exposures of British non-smokers to Environmental Tobacco Smoke, ETS, through a variety of inter-related measures. There are two main reasons for this investigation. The first is that, although considerable data exist quantifying levels of various constituents of ETS in fixed environments, there is relatively little data describing typical total daily exposures. The second is that much of the existing personal exposure data rely on measures of cotinine, a metabolite of nicotine in the body fluids of non-smokers. The accuracy of this measure has been questioned and this study proposes to examine the relationship between levels of cotinine and measures of chemical exposure to several ETS constituents and to questionnaire responses.

The study would randomly select around 300 non-smokers. Each subject would be investigated for exposure to ETS over a 24 hour period. The measures would be a time-activity diary, a post-sampling questionnaire on perceived exposure, salivary cotinine levels (pre- and post-monitoring period) and personal exposures to nicotine and to particulates. The particulate sample would be analysed for ultra-violet, fluorescence and solanesol content as assessments of the contribution of ETS to the total particulates collected. It is anticipated that such a study would prove information useful to the determination of the extent of ETS exposure and to the assessment of best measures of such exposure.

K. Phillips — Signature, Principal Investigator

12 August 1992 — Date

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CENTER FOR INDOOR AIR RESEARCH
1099 WINTERSON ROAD SUITE 280 LINTHICUM MD. 21090
(410)684-3777 FAX (410)684-3729

APPLICATION FOR RESEARCH CONTRACT

1. PRINCIPAL INVESTIGATOR. NAME. TITLE. TELEPHONE # AND MAILING ADDRESS.

Tel: 44 423 500011
Fax: 44 423 569565

(A) Mr Keith Phillips — NAME
(B) Manager — TITLE
(C) — TELEPHONE #/FAX #
(D) Analytical Chemistry — DEPARTMENT
(E) Hazleton UK — INSTITUTION
(F) Otley Road, Harrogate — MAILING ADDRESS
(G) North Yorkshire HG3 1P — STATE/ZIP

2. PROJECT TITLE. Determination of personal exposures to Environmental Tobacco Smoke in British non-smokers

3. KEY WORDS. PLEASE PROVIDE THREE (3) KEY WORDS WHICH WILL BE USED AS REFERENCE HEADINGS. Environmental Tobacco Smoke

4. INSTITUTION. NAME AND ADDRESS OF INSTITUTION RESPONSIBLE AND ACCOUNTABLE FOR DISPOSITION OF FUNDS AWARDED ON THE BASIS OF THIS APPLICATION.

(A) Hazleton UK — INSTITUTION
(B) Otley Road — STREET ADDRESS
(C) Harrogate — CITY
(D) North Yorkshire HG3 1PY — STATE/ZIP

5. LOCATION. LIST LOCATION WHERE RESEARCH WILL BE CONDUCTED *IF OTHER THAN INSTITUTION IDENTIFIED IN #4 ABOVE.*

(A)

(B)

6. INCLUSIVE DATES AND TOTAL COSTS OF THIS SPECIFIC PROJECT RELATED TO EACH 12 MONTH PERIOD IF MORE THAN ONE YEAR IS REQUIRED TO COMPLETE PROJECT. SUMMARIZE FROM BUDGET PAGE. ITEM 12(I). IT MUST BE UNDERSTOOD THAT AWARDS FOR 2ND AND 3RD PERIODS ARE DEPENDENT ON CENTER APPROVAL OF CONTINUATION APPLICATION.

	INCLUSIVE DATE			TOTAL COST
(A) 1ST 12 MONTH PERIOD	September 1992	THRU	August 1993	125,000-00 POUNDS STERLING
(B) 2ND 12 MONTH PERIOD IF REQUIRED	-	THRU	-	$
(C) 3RD 12 MONTH PERIOD IF REQUIRED		THRU		$

7. INSTITUTIONAL OFFICER. NAME. TITLE AND TELEPHONE NUMBER OF INDIVIDUAL AUTHORIZED TO SIGN FOR THE INSTITUTION IDENTIFIED IN #4 ABOVE. IT IS UNDERSTOOD THAT THE OFFICER, IN APPLYING FOR A CONTRACT, HAS READ AND FOUND ACCEPTABLE THE CENTER'S MANAGEMENT OF RESEARCH CONTRACTS AND CONTRACT ADMINISTRATION POLICY. (other than the payment schedule)*

(A) Mr M Wilson — NAME
(B) Contracts Administrator — TITLE
(C) 44 423 500011 — TELEPHONE
(D) M. Wilson — SIGNATURE OF INSTITUTIONAL OFFICER
(E) 7 September 1992 — DATE

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(b) Literature
(c) Identification of gaps in proposed researc area
(d) Project importance

XXXXXXXXXXXXXX

* APPEND AS MUCH MATERIAL AS REQUIRED; TYPE, SINGLE SPACE, USE 8-1/2" X 11" WHITE PAPER AND LABEL EACH SHEET WITH NAME OF THE PRINCIPAL INVESTIGATOR IN THE UPPER RIGHT HAND CORNER AND PAGE NUMBER AT THE BOTTOM. CONSECUTIVELY NUMBER EACH ADDENDUM BEGINNING WITH PAGE 5. DO NOT INSERT PAGES BETWEEN PAGES 1 AND 6, E.G., 2A, 2B, 3A, ETC. INCLUDE NINE COPIES AND AN ORIGINAL. IF SENDING PHOTOGRAPHS, INCLUDE 2 ORIGINAL SETS. NOTE: EACH OF THE NINE COPIES *MUST* BE PLACED IN A BINDER PER MAILING INSTRUCTIONS.

* Please see preferred method of payment on Hazleton Quotation.

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See page 3.

12. BUDGET. Detailed specific needs for the first 12-month period. Estimate category sub-totals for 2nd and 3rd periods, if required. Append justifications.

(a) Salaries. List personnel by name and title. Indicate individuals % time to be spent on this project.	$ 1st period	$ 2nd period	$ 3rd period
% Professional:			
% Technical:			
% Other:			
Fringe benefits payable at institution's rate of %			
Category (a) Sub-Total			
(b) Consultants (per diem, travel & expenses):			
Category (b) Sub-Total			
(c) Supplies & Expense: Consumables (by category)			
Animals and related costs			
Other expenses (itemize)			
Category (c) Sub-Total			
(d) Travel Expenses:			
Category (d) Sub-Total			
(e) Alterations and Renovations			
Category (e) Sub-Total			
(f) Sub-contracts			
Category (f) Sub-Total			
(g) Equipment			
Category (g) Sub-Total			
(h) TOTAL DIRECT COSTS			
(i) Indirect costs not to exceed 25% of the sum of (a) thru (f):			
(j) TOTAL PROJECT COSTS			

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13. **BUDGET**

	Pounds Sterling
Pilot Study (10 volunteers)	7,500
Main Study (minimum of 280 volunteers)	
• Volunteer recruitment, administration, reimbursement	24,500
• Collection/delivery of kits; equipment maintenance	9,500
• Analytical phase, to include method development/ validation and routine analysis of samples for total particulates, nicotine, UVPM/FPM, solanesol and salivary cotinine	75,000
• Prepare a report and a manuscript for publication	8,000
TOTAL	**125,000**

14 IX. BIOGRAPHICAL SKETCH of all professional personnel listed in 12(a). Append. Please include the following: Name, title, education, scientific field, major research interest, research and/or professional experience and publications. (Limit list of publications to the 20 most important and/or relevant.)

See Appendix C

15 IX. a) Are HUMAN SUBJECTS to be used in this research? _____ Yes _____ No
If yes, attach Institutional Review Board approval for procedures involving human subjects.

See Appendix D

b) Are LABORATORY ANIMALS to be used in this research? _____ Yes _____ No
If yes, attach Institutional Animal Care and Use Committee approval for procedures involving animals.

Not applicable

16 IX. SIGNATURE OF PRINCIPAL INVESTIGATOR: It is understood that the applicant in applying for a Contract has read and found acceptable the Statements of Policy and Terms Under Which Project Contracts Are Made appearing in the application package. (other than payment schedule)

K. Phillips 12 August 1992

Signature of Principal Investigator Date

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8 AIMS

The broad objective of the proposed work is to determine, through a variety of inter-related measures. the extent of exposure to ETS in typical British non-smokers.

The specific aims of the project are as follows:

1. To determine, in non-smoking British volunteers, the range and median levels of 24 hour exposure to nicotine and to ETS-related particulates.

2. To assess the contribution of exposure to ETS from different environments such as homes, the workplace and leisure and travel situations.

3. To assess whether non-smokers who are married to smokers have significantly higher exposures to ETS than non-smokers married to non-smokers.

4. To evaluate the extent of correlation between the different methods of exposure determination; questionnaires, salivary cotinine measures and personal monitoring of exposures to airborne constituents.

9. SIGNIFICANCE OF PROPOSED WORK

a) Background

Two approaches have been used to assess whether there is any risk associated with exposure to ETS. One is based on epidemiology and the other based on the quantities of smoke constituents to which non-smokers are exposed. Further information is required on typical exposure levels in order to address questions relating to epidemiology studies and to obtain a better assessment of how much ETS people are exposed to.

Most of the information about the exposure of non-smokers to ETS is based on measurements of ETS levels in locations such as homes, offices and restaurants with assumptions about the time spent in these locations. There have been several such studies, particularly in the USA, but not enough to characterise properly the range of ETS exposure of non-smokers. It is, therefore, important to obtain further information for a variety of other situations, including different countries with various climates and lifestyles.

Surprisingly, there have, until recently, been few attempts to measure exposure of people directly as they go about their normal lives, moving from location to location, even though this approach should provide more realistic results than those calculated from ETS levels in various locations. Although this personal monitoring technique has been common practice in the industrial hygiene field for several years, it is only recently that the analytical methodology has been refined sufficiently to allow ETS measurements to be carried out by this approach. A few ETS exposure studies of this type have now been completed or are underway.

Nevertheless, further studies in a variety of countries are still required in order to obtain sufficient information with which to address some of the important ETS issues.

Although levels of both nicotine and ETS particles have been determined in several studies of locations, personal monitoring studies have tended to measure nicotine but not particles. In view of the limitations of nicotine as a marker for ETS and the importance often attached to particles, there clearly is a need for complementary personal monitoring studies in which ETS particles are also measured, especially now that the UVPM (ie. ETS particulate matter measured by ultra-violet light), FPM (ie. ETS particulate matter measured by fluorescence) and solanesol methods are available for estimating the ETS contribution to total particles.

A criticism of existing epidemiological studies of ETS is that they failed to include a direct measure of exposure level. Spousal smoking has frequently been used as an index of exposure in these studies but the validity of this approach is open to question. It is, therefore, important to determine whether reported extent of spousal smoking correlates with directly measured exposure. For the same reasons, it would be useful to determine how well directly measured ETS exposure can be predicted by questionnaire or by measurements of salivary cotinine since these approaches are also used as an alternative to direct measurements. It would also be useful to establish how peoples' personal assessment of their exposure compares with their measured exposure.

Smoking bans are being introduced in the workplace and in various public leisure and travel situations. It would be helpful to obtain further information on the extent of exposure in these situations to assess how each contributes to overall exposure.

The Study proposed here will help to address these issues.

b) **Literature**

Perhaps the most extensive published evaluation of data related to ETS exposure is the monograph recently published by Guerin et al (Guerin, Jenkins and Tomkins, The Chemistry of Environmental Tobacco Smoke: Composition and Measurement, Lewis Publishers Inc. 1992). In this review of the existing literature, fourteen field studies of nicotine levels and twenty three field studies of particulate levels were tabled. Only one these studies referred to data acquired in the United Kingdom and so there is little to base comparisons in the literature between the predominately US based literature and a United Kingdom situation.

The same monograph briefly discusses the literature related to personal monitoring and biomarker assays. One personal monitoring study (Proctor et al, Environmental International 17, 287-297) measured personal exposures to nicotine and measured salivary cotinine levels in non-smoking British women. This study suggested a lack of correlation between cotinine and nicotine exposure levels. However, the study was small (50 subjects) and made no assessment of particulate exposures. US data on particulates (Spengler et al, Environmental Science and Technology, 19, 700-706, 1985) reported that 24 hour exposures to particulates were around 40 ug/m3 higher in those living in smokers' homes compared to non-smokers homes'. However, these researchers used comparative location techniques rather than chemical apportionation to determine the ETS contribution to particulates.

c) **Identification of gaps in proposed research area**

Our proposal addresses several gaps in the literature pertaining to the issue of population exposure to ETS. These are:

1. The sparsity of data specific to the United Kingdom. As far as we are aware there is only one UK based published study that has attempted to resolve the issues addressed in our proposal. Because of this it is uncertain whether the larger US database can be applied to the UK.

2. Little or no data exist on particulate exposure directly related to ETS as measured by chemical apportionation techniques.

3. The comparison of exposure assessment techniques (questionnaires versus chemical monitoring versus biomarker measurements) has rarely been addressed in studies measuring more that two of these comparative measures. The proposed study would compare six different measures (questionnaire, nicotine exposure, UV-PM exposure, Fluorescence-PM exposure, solanesol and salivary cotinine).

d) **Project importance**

Several agencies are currently considering the potential effects of exposure to ETS. In the United Kingdom, the Independent Scientific Committee on Smoking and Health stated it its Fourth report published in 1988 that it is recognised that the whole area of investigation of the composition and concentration of ETS is a difficult one and that it would keep the issue under review as new research findings became available. UK specific data would presumably be of value to this committee. On a broader basis, the investigation should prove useful in terms of an example of the use of personal monitoring techniques for investigating exposures to substances found in the environment.

10 PRELIMINARY STUDIES

a) Feasibility of the proposed research

A novel active monitoring device, which allows the simultaneous collection of airborne nicotine and particulates has been devised for this experiment. The effectiveness of this device has been evaluated in controlled experiments and we are confident that the collection technique will appropriately represent the personal exposures. The design and function of this device is described fully in the experimental plan. Apart from this, all of the methods proposed are standard and appear in the peer-reviewed literature.

b) Qualifications of investigator

The curriculum vitae of all the key investigators are appended to this proposal. The Institution, Hazleton UK, is experienced both in subject interview techniques and in the analysis if environmental and biological samples.

A profile of the company is attached to this proposal.

11 EXPERIMENTAL PLAN

a) Design

The following is a brief description of the study design. 300 smokers will be randomly selected from an existing database of 11,000 subjects held by GHBA/Hazleton Clinics in Leeds UK.

Either five or six volunteers will be studied each day such that eleven subjects will be studied every two days, including weekends. Each volunteer will be monitored for a continuous period of 24 hours.

The volunteers will all be from Yorkshire in the North of England, and will be selected to be representative in terms of age, sex and locality (urban/rural).

Their exposure to nicotine, TSP, UV-PM (particulates measured by UV light) F-PM (particulates measured by fluorescence) and solanesol will all be monitored.

At the beginning and the end of the monitoring period, saliva samples will be taken. The volunteers will maintain a diary throughout the monitoring period. A questionnaire will be completed at the end of the 24 hour period.

Prior to the start of the main study (approximately 3 to 4 weeks) a "pilot study" or trial will be conducted using ten volunteers. The purpose of the trial is to assess all aspects of the main study including collection, analysis and questionnaire completion and to highlight any problems that might occur in the main study.

b) Methods

i) Subjects

300 non-smokers will be randomly selected from an existing database of 11,000 subjects held by GHBA/Hazleton Clinics in Leeds, UK. All volunteers are to be non-smokers aged between 20 and 60 years of age. Subjects will reside in the Leeds and Harrogate area in the North of England and they will be distributed based on age, sex and locality (urban/rural).

A pre-acceptance questionnaire will be used to select an excess of volunteers so that in the event of drop-outs suitable replacement candidates can be selected. The volunteers will be provided a financial incentive for their involvement in the study.

ii) **Sample delivery and collection**

A minimum of 280 volunteers results are required. To acheive this, five or six subjects will be monitored daily producing 11 samples every two days over a period of fourteen consecutive days. Thus 70 results will be obtained in a two week period. This regime will be repeated on three consecutive occasions.

The personal monitors will be delivered to the volunteers at pre-determined locations and times and will be collected as close as possible to 24 hours later. The monitor pump will be turned on and off by the investigators and not by the subjects. Times of start and finish, as well as recorded cycles of the pumps, will be recorded.

Saliva samples will be taken from each subject at the beginning and at the end of the 24 hour sampling period.

Questionnaires (see appendix A) will be completed by the investigator who will ask a series of pre-determined questions, coded for later analysis. These questions will be asked at the end of the sampling period. The volunteers will also carry a time-activity diary in order to record observations throughout the monitoring period.

iii) **Collection and analysis of airborne nicotine and particulates**

The collection of these analytes relies upon the use of a compact collection system which is worn by the subject in order to sample the air to which he/she is exposed. It consists of two filters in series connected to a sampling pump. The first filter collects the total particulates and the second, which is acid-treated, traps nicotine vapour.

Air is drawn through the filters by a small, quiet, battery powered pump which is concealed in a small bag worn at the subject's waist level. The pump is set at a flow rate of 139 ml/min so that a total volume of 200 litres is drawn through the pump during the 24 hour monitoring period.

The filter holder is attached to a rigid wire "necklace" which holds the monitor in place and allows ease of removal. A clip will be provided as an alternative to the necklace.

During periods of sleep or bathing the monitor will be taken off but be placed close to the subject. Such events will be noted in the time-activity diary.

A detailed description of the monitor and of the procedures designated for this study are given in Appendix B of this proposal (which details all of the methodology).

In brief, the analysis of the nicotine contained on the acid treated pad involves extraction into dis-isopropyl ether (DIPE) (containing 0.1m/l triethylamine and 2.0 mg/l N-ethylnornicotine (internal standard) from sodium hydroxide which is used to basify the filter.

The DIPE extract is then analysed by capillary gas chromatography with nitrogen specific detection.

The total suspended particulate concentration is determined gravimetrically by the difference in weights of the front teflon pad before and after sampling.

After weighing, the pad is extracted in methanol in order to determine UV-PM, F-PM, solanesol and any residual nicotine that might have been trapped on the front pad. Again all of these procedures are detailed in Appendix B.

iv) **Collection and analysis of saliva**

Saliva will be collected from each subject immediately before and after each monitoring period. This will be achieved by the subject chewing on a dental swab for around a minute. The swab is then returned to the laboratory sealed in its salivette container.

The saliva is recovered by high speed centrifuge for two minutes. Cotinine and N-ethylnorcotinine (internal standard) are extracted from the saliva and the extract analysed by GC with nitrogen specific detection.

v) **Detection limits**

Under the sampling regime described, the detection limits for the various analytes are expected to be as follows:

Total particulates	20 ug/m3 as ETS particulates
UV-PM	5 ug/m3 as ETS particulates
F-PM	5 ug/m3 as ETS particulates
Solanesol	10 ug/m3 as ETS particulates
Nicotine	0.5 ug/m3
Salivary cotinine	0.5 ng/ml

vi) **Quality Control**

The study will be performed where appropriate in accordance with the Good Laboratory Practice provided as guidelines of the UK Department of Health compliance programme (1989). Where appropriate all work will be performed under Hazleton's standard operating procedures.

Any deviations from the protocol will be recorded as a file note against the raw data and highlighted in the final report.

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c) **Analysis of data**

Subject information and corresponding analytical data will be compiled in a database as the study progresses.

Computation of means and ranges for each of the analtyes and correlations between the different analytes will be achieved through standard statistical procedures.

d) **Interpretation of the results**

The results will be reported both as a detailed research findings report to the Center for Indoor Air Research and as a manuscript for publication.

e) **Timetable of investigation**

Should approval be received, the pilot phase of the study could begin within one month. Field sampling would occur over a period of around two and one half months. Data analysis and reporting is expected to be complete three months after the completion of sampling.

12 AVAILABLE FACILITIES AND RESOURCES

Hazleton UK is the European Headquarters of Hazleton Corporation, a wholly owned subsidiary of Corning Laboratory Services Inc. The company provides a wide range of product development and safety evaluation services to the pharmaceutical, agrochemical and chemical industries.

The laboratories at Harrogate, which occupy 185,000 square feet on a 20 acre site, are engaged in general and reproduction toxicology, molecular toxicology, metabolism and pharmacokinetics and biological and chemical analysis. The 50 bed GHBA/Hazleton Clinic, Leeds, undertakes clinical pharmacology studies in healthy volunteers and a variety of patient population groups.

All studies conducted by Hazleton and GHBA satisfy requirements for Good Laboratory and Good Clinical Practices (GLP and GCP) respectively.

Of the 625 staff, 159 are degree level and 39 doctorate level. Five percent of time is devoted to training, as part of the company's Total Quality Management programme.

The modern analytical laboratories are particularly well equipped to undertake the proposed study and the Principal Investigator has direct experience of tobacco smoke analysis studies.

HLR AIR QUALITY SURVEY QUESTIONNAIRE
SECTION 1

INTERVIEWER'S NAME.....

DATE.....

SUBJECT'S NAME.....

AGE.....

MALE/FEMALE.....

MARRIED/PARTNER/SINGLE.....

LIVING WITH SPOUSE/PARTNER.....

ADDRESS.....

POST CODE.....

OCCUPATION.....

OCCUPATION POST-CODE.....

DATE OF MONITORING START.....

TIME OF MONITORING START.....

TIME OF MONITORING END.....

PUMP NUMBER.....

PUMP COUNTS END.....

PUMP COUNTS START.....

PUMP COUNTS DIFFERENCE.....

MONITOR NUMBER.....

WAS THE MONITOR KEPT IN YOUR VICINITY AT ALL TIMES? .....

WAS THE MONITOR INTERFERED WITH BY ANYBODY?.....

DID ANYONE DELIBERATELY BLOW SMOKE INTO THE MONITOR?.....

TEMPERATURES AND WEATHER IN THE LEEDS/HARROGATE AREA *(To be recorded by Hazleton)*

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HLE AIR QUALITY SURVEY QUESTIONNAIRE
SECTION 2

The purpose of this Section is to discover whether any factors during the monitoring period would have influenced the general air quality and hence the quantities of material collected on the filters.

DID YOU SPEND ANY TIME IN A DUSTY ATMOSPHERE?.....

DID YOU SPEND ANY TIME NEAR HEAVY TRAFFIC?.....

IS YOUR HOME NEAR A BUSY ROAD?.....

WHICH AEROSOL SPRAYS DID YOU USE?

.....

WHAT TYPE OF HEATING IS USED IN YOUR HOME?.....

DID YOU USE THE VACUUM CLEANER OR DID ANYONE ELSE USE IT WHILE YOU WERE THERE?.....

DID YOU DO, OR WERE YOU NEAR, ANY PAINTING OR DECORATING?.....

DID YOU DO, OR WERE YOU NEAR, ANY COOKING?.....

WAS ANY FRYING DONE WHILE YOU WERE AT HOME?.....

DO YOU HAVE ANY OTHER OBSERVATIONS ABOUT AIR QUALITY DURING THE TEST PERIOD?

HLE AIR QUALITY SURVEY QUESTIONNAIRE
SECTION 3

The purpose of this Section is to discover the subject's perception of exposure to ETS and whether the monitoring period was typical of the subject's normal exposure level.

HOW DO YOU RATE YOUR EXPOSURE TO TOBACCO SMOKE DURING THE TEST PERIOD?

None Low Moderate High Very High

HOW DO YOU RATE YOUR EXPOSURE TO TOBACCO SMOKE DURING THE SAME PERIOD IN NORMAL WEEKS?

None Low Moderate High Very High

HOW DO YOU RATE YOUR EXPOSURE TO TOBACCO SMOKE ON AVERAGE OVER THE LAST SIX MONTHS?

None Low Moderate High Very High

HOW DID YOUR EXPOSURE TO TOBACCO SMOKE DURING THE TEST PERIOD COMPARE WITH YOUR AVERAGE EXPOSURE LEVEL OVER THE LAST SIX MONTHS?

Much less than average.

Less than average.

Fairly typical of average exposure.

More than average.

Much more than average.

HLE AIR QUALITY SURVEY QUESTIONNAIRE
SECTION 4

The purpose of this Section is to assess how much exposure to tobacco smoke occurred in different situations during the test period.

HOW MANY HOURS DID YOU SPEND AT HOME?.....

WHAT TYPE OF ACCOMMODATION IS YOUR HOME?

House Flat Caravan Other.....

HOW WELL VENTILATED IS THE ACCOMMODATION?

Good Moderate Poor

HOW MANY PEOPLE LIVING AT YOUR HOME ARE SMOKERS?.....

HOW MANY PEOPLE LIVING AT YOUR HOME SMOKE AT HOME?.....

DID ANYONE SMOKE IN YOUR HOME DURING THE TEST PERIOD?.....

FOR HOW MANY HOURS AT HOME WERE YOU IN THE SAME ROOM AS SOMEONE SMOKING DURING THE TEST PERIOD?.....

HOW DO YOU RATE YOUR EXPOSURE TO TOBACCO SMOKE DURING THESE HOURS AT HOME IN THE PRESENCE OF SMOKING?

None Very Low Low Moderate High Very High

HOW MANY HOURS DID YOU SPEND AT LEISURE?.....
(This does not include leisure at home)

Pub Restaurant Club Cinema Church Sport Education Visiting Others.....

FOR HOW MANY OF THESE HOURS AT LEISURE WERE YOU IN THE PRESENCE OF SMOKING?.....

HOW DO YOU RATE YOUR EXPOSURE TO TOBACCO SMOKE DURING THESE HOURS OF SMOKING?

None Very Low Low Moderate High Very High

HOW MANY HOURS DID YOU SPEND IN TRAVEL?.....

Car Bus Train Plane Motorcycle Cycle Walking Other.....

FOR HOW MANY OF THESE HOURS OF TRAVEL WERE YOU IN THE PRESENCE OF SMOKING?.....

HOW DO YOU RATE YOUR EXPOSURE TO TOBACCO SMOKE DURING THESE HOURS OF SMOKING?

None Very Low Low Moderate High Very High

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HLE AIR QUALITY SURVEY QUESTIONNAIRE
SECTION 4 (continued)

HOW MANY HOURS DID YOU SPEND AT WORK?.....
(ALL travel should be reported in the questions about travel)

HOW MANY HOURS AT WORK DID YOU SPEND INDOORS?.....

HOW MANY HOURS AT WORK DID YOU SPEND IN THE OPEN AIR?.....

HOW MANY HOURS AT WORK DID YOU SPEND TRAVELLING?.....

If any work is indoors
HOW GOOD IS THE VENTILATION IN THE MAIN AREA WHERE YOU WORK INDOORS?

Good Moderate Poor

If any work is indoors
IS THERE AIR CONDITIONING IN THE MAIN AREA WHERE YOU WORK INDOORS?.....

If any work is indoors
IS SMOKING PERMITTED IN THE MAIN AREA WHERE YOU WORK INDOORS?.....

If any work is indoors
IS THERE A SPECIAL AREA SET ASIDE FOR SMOKING?.....

If work is indoors
APPROXIMATE DIMENSIONS OF MAIN AREA AT WORK WHERE EXPOSURE TOOK PLACE
(Feet).....

FOR HOW MANY HOURS AT WORK DURING THE TEST PERIOD WERE YOU IN THE PRESENCE OF SMOKING?.....

HOW DO YOU RATE YOUR EXPOSURE TO TOBACCO SMOKE DURING THESE HOURS OF SMOKING?

None Very Low Low Moderate High Very High

If any exposure to ETS reported in Section 4
WHAT PERCENTAGE OF YOUR TOTAL EXPOSURE TO TOBACCO SMOKE IN THE TEST PERIOD OCCURRED

At home

At work

In travel

During leisure

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HLR AIR QUALITY SURVEY QUESTIONNAIRE
SECTION 5

The purpose of this Section is to assess the effects of Spousal Smoking and of living with smokers.

HOW MANY CIGARETTES DOES YOUR SPOUSE/PARTNER NORMALLY SMOKE PER DAY? .....

HOW MANY CIGARETTES DOES YOUR SPOUSE/PARTNER NORMALLY SMOKE IN YOUR PRESENCE PER DAY?.....

DID YOUR SPOUSE/PARTNER SMOKE A PIPE OR CIGARS IN YOUR PRESENCE DURING THE TEST PERIOD? .....

FOR HOW MANY HOURS DID YOUR SPOUSE/PARTNER SMOKE IN YOUR PRESENCE DURING THE TEST PERIOD?.....

HOW DO YOU RATE YOUR EXPOSURE TO YOUR SPOUSE'S/PARTNER'S TOBACCO SMOKE DURING THE TEST PERIOD?

None Very Low Low Moderate High Very High

FOR HOW MANY HOURS DID OTHER MEMBERS (NOT INCLUDING SPOUSE/PARTNER) OF YOUR HOUSEHOLD SMOKE IN YOUR PRESENCE AT HOME DURING THE TEST PERIOD?.....

FOR HOW MANY HOURS DID OTHER MEMBERS (NOT INCLUDING SPOUSE/PARTNER) OF YOUR HOUSEHOLD SMOKE IN YOUR PRESENCE NOT AT HOME DURING THE TEST PERIOD?.....

HOW DO YOU RATE YOUR EXPOSURE TO TOBACCO SMOKE FROM OTHER MEMBERS OF YOUR HOUSEHOLD (NOT INCLUDING SPOUSE/PARTNER) DURING THESE HOURS OF SMOKING?

None Very Low Low Moderate High Very High

FOR HOW MANY HOURS DID VISITORS TO YOUR HOME SMOKE DURING THE TEST PERIOD IN YOUR PRESENCE?.....

HOW DO YOU RATE YOUR EXPOSURE TO TOBACCO SMOKE FROM VISITORS TO YOUR HOME DURING THESE HOURS OF SMOKING?

None Very Low Low Moderate High Very High

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HLE AIR QUALITY SURVEY QUESTIONNAIRE
SECTION 6

The purpose of this Section is to discover the reported smoking habits of the subjects, all of whom should theoretically be non-smokers.

HOW MANY CIGARETTES DID YOU SMOKE DURING THE TEST PERIOD?.....

HOW MANY CIGARETTES DID YOU SMOKE DURING THE LAST WEEK?.....

HOW MANY CIGARETTES DID YOU SMOKE DURING THE LAST YEAR?.....

FOR HOW MANY YEARS HAVE YOU SMOKED DURING YOUR LIFE?.....

WHAT WAS THE AVERAGE NUMBER OF CIGARETTES SMOKED PER DAY DURING THESE YEARS OF SMOKING?.....

WHEN DID YOU LAST SMOKE?.....

COMMENTS ABOUT THE TEST PERIOD BY THE SUBJECT.

COMMENTS BY THE INTERVIEWER.

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UK PERSONAL MONITORING STUDY

METHODOLOGY

SUMMARY

Subjects' exposure to ambient nicotine and TSP from all sources is measured over a 24 hour period using a personal monitoring technique.

The nicotine and TSP are drawn through two filters held in a small filter holder using a battery-operated pump.

TSP collected on the front filter is estimated gravimetrically and the ETS contribution to TSP is estimated by UV, fluorescence and solanesol measurements on a methanol extract of the filter. Nicotine, collected partly by the front filter and mainly by the second filter, is determined by gas chromatography.

Saliva samples are taken from subjects at the beginning and end of the 24 hour monitoring period and the cotinine content is determined by gas chromatography.

OUTLINE PROCEDURE

PREPARATION OF THE PERSONAL MONITOR FOR A MONITORING SESSION

CLEAN THE FILTER HOLDER AND ASSOCIATED PARTS

PREPARE THE ACID TREATED FILTER

WEIGH THE TEFLON FRONT FILTER

ASSEMBLE THE FILTER HOLDER.

CONNECT THE PUMP AND CHECK THAT THE HOLDER IS LEAK-TIGHT.

SET THE PUMP FLOW RATE AND NOTE THE PUMP COUNTER READING.

APPLY SECURITY TAG

SEAL THE FILTER HOLDER WITH CAP

ASSEMBLE A FILTER AS A BLANK CHECK

OUTLINE PROCEDURE

STARTING THE MONITORING SESSION

TRAIN SUBJECT ON USING THE MONITOR AND USE OF THE DIARY

TAKE SALIVA SAMPLE

NOTE THE PUMP COUNTER READING

REMOVE CAP FROM FILTER HOLDER

CHECK THE SECURITY TAG

START PUMP AND RECORD THE PUMP START TIME AND DATE

OUTLINE PROCEDURE

ENDING THE MONITORING SESSION

THE MONITORING SESSION WILL END 24 HOURS AFTER IT STARTS

SWITCH OFF THE PUMP

NOTE THE PUMP COUNTER READING AND THE PUMP STOP TIME

CHECK THE SECURITY TAG

SEAL THE FILTER HOLDER WITH CAP

TAKE SALIVA SAMPLE

COMPLETE THE QUESTIONNAIRE AND COLLECT THE DIARY

OUTLINE PROCEDURE

ANALYSIS OF THE COLLECTED SAMPLES

RECORD THE WEATHER CONDITIONS FOR THE MONITORING PERIOD

CHECK INTEGRITY OF SECURITY TAG

NOTE THE PUMP COUNTER READING

CHECK THE HOLDER IS LEAK TIGHT

CHECK PUMP FLOW RATE

DISMANTLE THE HOLDER

WEIGH THE TEFLON FRONT FILTER TO DETERMINE TSP

EXTRACT THE TEFLON FILTER WITH METHANOL. ANALYSE FOR UVPM, FPM, SOLANESOL AND NICOTINE

ANALYSE THE ACIDIC FILTER FOR NICOTINE

ANALYSE THE FILTER HOLDER BLANK FOR THE SAME ANALYTES

ANALYSE THE SALIVA SAMPLES FOR COTININE

EQUIPMENT

1. MICROBALANCE

Sartorius model M3P (6 decimal place) or equivalent. The balance should be mounted on a very stable surface and situated in a temperature controlled laboratory away from strong draughts.

The microbalance should stand on an earthed antistatic mat. During microweighing, the operator should be connected to this mat via an antistatic wrist-band strap. This arrangement eliminates static charge build-up arising from the operator and ambient conditions during microweighing.

2. CHECK-WEIGHT FOR MICROBALANCE

A length of platinum or nichrome wire weighing about 25mg, ie the same weight as a Millipore filter. This should be bent into a convenient shape so that it is easily picked up with tweezers and easily placed on and removed from the balance pan or weight cradle.

The check weight should be stored on aluminium foil in a closed container when not in use.

3. RADIOACTIVE SEALED SOURCE STATIC ELIMINATOR

BAR-TYPE POLONIUM-210 (approximately 148 M Bcq)

CATALOGUE NUMBER: PDV 1

SUPPLIER : Amersham International PLC, Buckinghamshire, England

Polonium-210 radioactive static eliminators have a working lifetime of approximately one year.

4. FLUOROPORE MEMBRANE FILTERS

DIAMETER: 25mm

PORE SIZE: 1 MICROMETER

CATALOGUE NUMBER: FALP 02500

SUPPLIER: Millipore U.K. LTD, Hertfordshire, England.

12. EQUIPMENT FOR SALIVARY COTININE DETERMINATION.

GC, on-column injector, NPD, integrator.

13. GLASSWARE

Conical flasks, 15ml, with screw top fitting and screw caps with Teflon inner lining.

Test tubes, 15ml, with screw top fitting and screw caps with Teflon inner lining.

REAGENTS

Methanol: (Romil) Super Purity, 99.9% minimum

Di-isopropyl ether (DIPE): (Romil) Super Purity, 99.9% minimum

DIPE solvent. Containing (1) 0.1 ml/litre triethylamine

(2) 2.0 mg/litre N-ethylnornicotine.

Solanesol: (Sigma), 90%+. Purity checked against purified solanesol (98%+)

Nicotine: Double distilled 99%+

N-ethyl nornicotine: Double distilled 99%+

Cotinine: (Lancaster Synthesis) 98%

N-ethyl norcotinine 98%

Scopoletin: (Aldrich) 95%

2,2',4,4' Tetrahydroxybenzophenone: (Aldrich) 97%

Triethylamine: (BDH) 99.5%

PREPARING THE PERSONAL MONITOR

CLEANING AND PREPARATION OF THE HOLDER

Wash the filter holder body and the Teflon spacers in hot water containing a little detergent. Rinse these parts in distilled water and then in methanol.

Dry all parts in an oven at 60C and store in a smoke-free area.

PREPARATION OF THE ACID-TREATED FILTER

Immerse the Pallflex filter in a fresh 4% solution of sodium bisulphate for 30 seconds. Remove the filter and allow excess liquid to drain off. Place the filter on a clean watch glass and allow it to dry for one hour in a dessicator. Store in the dessicator or a closed vessel until ready for use.

NB. These filters will absorb nicotine from the atmosphere.

These filters should be prepared fresh for each day's use.

WEIGH THE TEFLON FRONT FILTER

Ensure the balance has gone through its daily calibration check and that the check-weight is within 1ug of the last check and 2ug of the expected value determined at the start of the project..

Attach the earthing strap to a wrist.

Handle the filter only with stainless steel tweezers and hold the filter under the static eliminator at a distance of 5cm for about 10 seconds before each weighing.

Weigh the filter five times or until five consecutive weighings are each within a total range of 3ug. Record the average weight of these five weighings.

Correct the filter weight by the difference between the check weight and its expected weight.

ASSEMBLE THE FILTER HOLDER

Using fine pointed stainless steel tweezers place the filters and Teflon spacers in the filter holder body as in the arrangement shown in FIG 1.

Note that the dull face of the Millipore filter and the rough, cream face of the Pallflex filter should face the air inlet.

Assemble the front face to the filter body ensuring that it fits over the rubber 'O' ring properly.

Screw on the locking ring firmly.

CONNECT THE PUMP AND CHECK THAT THE HOLDER IS LEAK-TIGHT.

Using a fully-charged pump, adjust the flow rate to 139 ml/min. Connect the pump inlet to the filter holder using the plastic tubing provided, ensuring a tight connection at the pump and the filter holder. The plastic tube should pass through the hole in the side of the belt bag.

Replace the tubing if a tight seal cannot be obtained.

Turn on the pump briefly and ensure that it is running properly. Cap the filter holder with the cap provided and check that the pump comes to a stop within 10 seconds. If the pump continues to run then the filter holder is not leak tight and should be checked.

SET THE PUMP FLOW RATE AND NOTE THE PUMP COUNTER READING.

Connect the outlet of the pump to the flow meter and check the pump flow rate is between 136 and 142 ml/min. This work should be done in a clean area where no smoking is allowed and air should not be pumped through the assembled filter for more than two minutes. The amount of contamination of the filter in this time is negligible.

Note how many pump strokes are recorded on the counter in a 1.00 minute period (approximately 250 depending on the individual pump). This figure will be used to check correct operation of the pump during the sampling period.

Cap the filter holder with its cap and note the pump reading.

Apply the security tag. Fit the pump into its bag and close the bag.

ASSEMBLE A FILTER HOLDER AS A BLANK CHECK

For each series of personal monitors assembled on a given day, assemble one blank holder. Attach a pump and set the flow rate in

the same way as for the other monitors. Cap the holder and retain until the group of personal monitors is returned for analysis.

STARTING THE MONITORING SESSION

TRAINING THE SUBJECT ON USING THE MONITOR AND USE OF THE DIARY.

Explain to the subject that the study is looking at air quality but do not mention that the primary purpose relates to cigarette smoke.

Show the subject how to wear the monitor and how to take it on and off when changing clothes or going to bed. Ask if he/she has any objection (eg. safety) to wearing the monitor with the 'necklace' and offer the use of safety pins if this is the case.

Ask the subject not to let the monitor interfere with normal behaviour.

Instruct the subject not to interfere with the monitor and not to let anyone else to interfere with it. Explain that tampering with the monitor will be detected and will result in loss of the subject's reward.

Provide the subject with the security card which explains that he/she is involved in an air quality study conducted by Hazleton.

Explain that the subject should remove the monitor when he/she goes to bed and place the monitor nearby such that the air inlet is not obstructed. If the pump is found to be too noisy during this time, the belt bag containing the pump can be placed in a larger bag or covered with a pillow. The filter holder must not be obstructed or covered in any way and the plastic tube connecting the pump to the filter holder must not be kinked.

Instruct the subject to complete the diary during each hour awake during the monitoring period. The main requirement is to record location, activity and factors which might affect air quality, including smoking.

TAKE SALIVA SAMPLE

Remind the subject that a saliva sample is required at the beginning and end of the sampling period. Reassure the subject if necessary that only a chemical test and no medical tests will be done on the sample.

Collect the saliva sample according to the procedure in Appendix 4.

Label the sample tube clearly with the subject's name and the date and time of sampling. Also note whether it is the 'pre-sample' or the 'post-sample'.

Transfer the saliva sample to a freezer as soon as possible and retain there until ready for use.

NOTE THE PUMP COUNTER READING

Open the belt bag and note the pump stroke counter reading. This should be recorded on Page 1 of the questionnaire.

REMOVE CAP FROM FILTER HOLDER

Remove the filter holder cap from the air inlet. Do not leave this cap with the subject.

FIT THE MONITOR, START THE PUMP AND NOTE THE PUMP START TIME

Attach the 'necklace' to the filter holder and fit the personal monitor to the subject. Safety pins can be used in place of, or in addition to, the necklace if necessary for a particular subject.

Switch on the pump and ensure that it runs at normal speed for at least 30 seconds. The pump start time should be recorded on Page 1 of the questionnaire.

Close the belt bag.

Confirm the appointment for the end of sampling period in 24 hours time.

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ENDING THE MONITORING SESSION

The monitoring session should end as close as possible to 24 hours after it started and certainly not at less than 23 hours or more than 25 hours.

SWITCH OFF THE PUMP

Open the belt bag and check that the pump is running normally. Switch off the pump and record the stroke counter reading and the switch-off time on Page 1 of the questionnaire.

Check the security tag.

Seal the holder with the cap.

Take a saliva sample by the same procedure as at the start of the monitoring session.

Label the sample tube clearly with the subject's name and the date and time of sampling. Also note whether it is the 'pre-sample' or the 'post-sample'.

Transfer the saliva sample to a freezer as soon as possible and retain there until ready for use.

COMPLETE THE QUESTIONNAIRE AND COLLECT THE DIARY

Do not explain to the subject that the main purpose of the study relates to cigarette smoke.

Complete the questionnaire as well as possible, making use of the diary to get answers which are as accurate as possible.

Collect the Diary from the subject and keep it together with the questionnaire.

ANALYSIS OF COLLECTED SAMPLES

RECORD THE WEATHER CONDITIONS FOR THE SAMPLING PERIOD

CHECK THAT THE SECURITY TAG IS INTACT.

If the tag is not intact and was not found to be intact by the interviewer at the end of the sample period, do not proceed with the analysis.

NOTE THE PUMP STROKE COUNTER READING

Note the pump stroke counter reading.

Check that the laboratory record of stroke counts corresponds with that of the interviewer for the start and end of the monitoring session.

Ensure that the stroke counts recorded during the sampling session are consistent with continuous sampling for 24 hours using the stroke count rate determined during setting the flow rate.

CHECK THE HOLDER IS LEAK TIGHT

With the filter holder inlet still capped, turn the pump on. The pump will stop within 10 seconds if the pump is leak tight.

CHECK THE PUMP FLOW RATE

Remove the filter holder cap and measure the pump flow rate by connecting the pump outlet to the flow meter. The pump flow rate should be close to the 139ml/min set prior to sampling and must be at least 120 ml/min.

Calculate and record the volume of the air sample collected in litres.

VA=(V1+V2)*T*/2000 litres.

Where:

V1=pump flow rate at the start of sampling in ml/min.

V2=pump flow rate at the end of sampling in ml/min.

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T=duration of the sampling period in minutes.

DISMANTLE THE HOLDER

Break the security tag and unscrew the locking ring.

Carefully remove the front face of the holder while keeping the holder upright to ensure that the filters staying place.

WEIGH THE TEFLON FRONT FILTER TO DETERMINE TSP

Carefully remove the Millipore filter from the holder with clean stainless steel tweezers.

Ensure the balance has gone through its daily calibration check and that the check-weight is within 1ug of the last check and 2ug of the expected value determined at the start of the project.

Weigh the filter by the same procedure as used when the filter was weighed before the sampling period.

Correct the filter weight by the difference between the check weight and its expected weight.

Calculate the weight of particulate matter collected on the filter from the difference in weight before sampling and after sampling.

Calculate the weight of particulate matter in ug collected per cubic metre.

TSP=(W2-W1)*1000/VA ug/m3

Where:

W1=weight of filter before sampling in ug.

W2=weight of filter after sampling in ug.

VA=volume of air sample collected in litres.

EXTRACT THE TEFLON FRONT FILTER AND TEFLON SEAL WITH METHANOL ANALYSE FOR UVPM, FPM, SOLANESOL AND NICOTINE

Place the Millipore filter and Teflon seal (See Figure 1) in a clean 15ml screw top conical flask.

Add 4.0ml of methanol.

Cap with a screw cap having a Teflon face and shake for 30 minutes.

Fill a 2ml autosampler vial with the methanol extract and cap.

This will be used for the UVPM, FPM and solanesol measurements (see Appendix 2)

Store the vial in the refrigerator, for upto a maximum of three days, until ready for use.

Pipette 1.0ml of the methanol extract into a screw topped 15ml test tube. Add 1.0 ml of DIPE (containing internal standard and triethylamine) and 4ml of 5N sodium hydroxide. Cap the tube with a Teflon-lined cap. Mix thoroughly for 10 minutes using a vortex mixer. Allow the phases to separate and then transfer at least 0.5ml of the upper (DIPE) layer to an autosampler vial and cap the vial. Take care not to transfer any of the aqueous phase.

This vial is used to measure nicotine collected on the first filter (see Appendix 3).

Store the vial in the refrigerator, for upto a maximum of three days, until ready for use.

ANALYSE THE ACIDIC FILTER FOR NICOTINE

Remove the first Teflon pad, the acid-treated Pallflex filter and the second teflon spacer from the filter holder using clean stainless steel tweezers. Transfer these to a screw-topped 15ml conical flask and add 1.0 ml of DIPE (containing internal standard and triethylamine) followed by 4ml of 5N sodium hydroxide. Shake the flask on a shaker for 30 minutes.

Transfer as much of the liquid as possible to a screw-topped 15ml test tube and cap the tube with a Teflon-lined cap.

Allow the phases to separate and then transfer about 0.5ml of the upper (DIPE) layer to an autosampler vial and cap the vial. Take care not to transfer any of the aqueous phase.

This vial is used to measure nicotine collected on the second filter (see Appendix 3).

Store the vial in the refrigerator, for upto a maximum of three days, until ready for use.

ANALYSE THE FILTER HOLDER BLANK FOR THE SAME ANALYTES

Dismantle the blank filter holder and carry out the same weighing and analyses as for the sample filter holders. Correct sample readings for any blank readings.

Blank readings should not exceed the following values:

Note: No significant change in filter weight should occur due to just assembling and dismantling the filter as in the blank holder. However, if a consistent weight change is found for the blank holders it will be necessary to correct for this on the sample weights. If this correction is more than 3ug then procedures should be reviewed.

ANALYSE THE SALIVA SAMPLES FOR COTININE

Analyse the samples according to the method in Appendix 4.

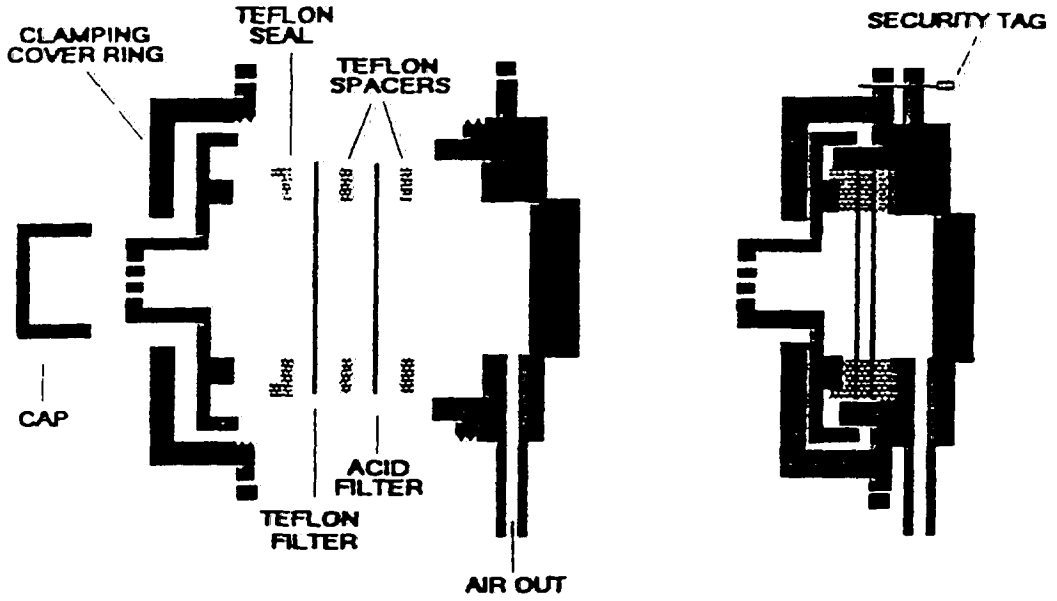

FIGURE 1A. FILTER HOLDER APART AND ASSEMBLED

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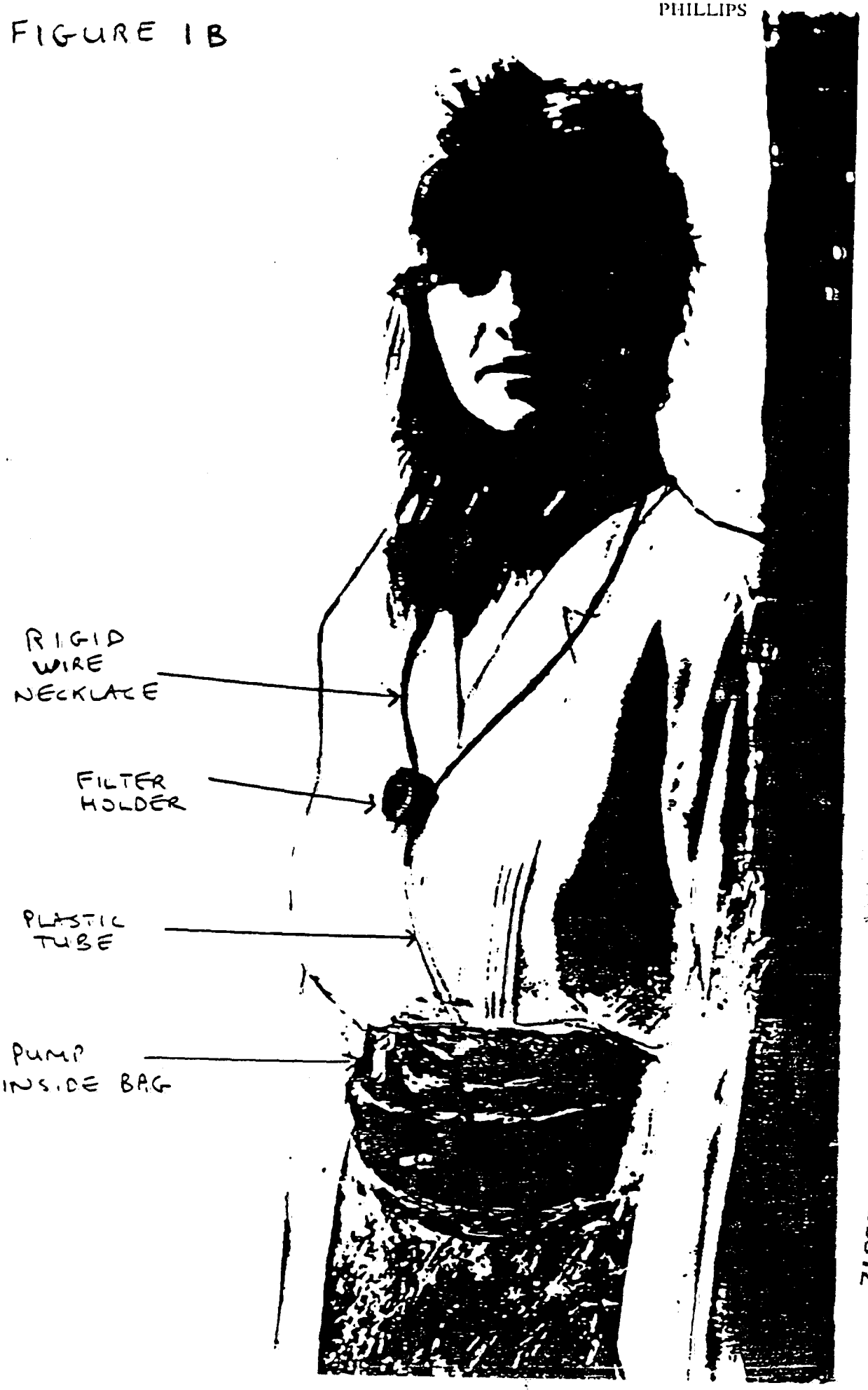
FIGURE 1B



APPENDIX 1

ESTIMATION OF UVPM AND FPM

PRINCIPLE

Particulate matter collected on a Millipore Teflon filter is extracted with methanol. UV and fluorescence measurements are made on the extract and these are compared to calibrations made using surrogate standards for ETS particulates.

The calibration of surrogate standards against ETS has been established in prior experiments using ETS generated in a Model Room using the same master calibration solutions.

APPARATUS

HPLC Pump capable of pumping methanol at 0.4ml/min.

UV detector set at 325nm.

Fluorescence detector with Excitation 300nm and Emission 420nm.

Thermostatted column oven set at 30C

Delay tube / restrictor: Empty stainless steel tube, 4m long, 0.33mm ID

Frit filter

Loop injector with 50ul loop.

The equipment is set up as a column-less HPLC system as shown in FIGURE 2.

REAGENTS

Methanol: (Romil) Super Purity, 99.9% minimum

Scopoletin: (Aldrich) 95%

2,2',4,4' Tetrahydroxybenzophenone: (Aldrich) 97%

CALIBRATION

1. UVPM

Prepare (by dilution from a stronger solution) a master solution containing 12.5mg of 2,2',4,4' tetrahydroxybenzophenone (THBP) in 2.5 litres of methanol (ie 5 ug/ml).

Store this solution in a 2.5 litre amber Winchester reagent bottle used previously to store methanol.

This solution is stable for at least six months when stored in a refrigerator.

Prepare calibration solutions by dilution of the master solution with methanol as follows:

CONCENTRATION ug/ml	DILUTION OF MASTER	EQUIVALENT UVPM ug/ml
2.5	50ml to 100ml	15·25 ≡ 305 µg/m³
1.0	20ml to 100ml	using latest conversion factor of 6·1
0.5	10ml to 100ml	
0.25	5ml to 100ml	
0.10	2ml to 100ml	
0.05	1ml to 100ml	

These diluted calibration solutions should be prepared fresh every week and stored in the refrigerator.

2. FPM

Prepare (by dilution from a stronger solution) a master solution containing 2.5mg scopoletin in 2.5 litres of methanol (ie 1ug/ml).

Store this solution in a 2.5 litre amber Winchester reagent bottle used previously to store methanol.

This solution is stable for at least six months when stored in a refrigerator.

Prepare calibration solutions by dilution of the master solution with methanol as follows:

CONCENTRATION ug/ml	DILUTION OF MASTER	EQUIV. FPM ug/ml
0.50	50 ml to 100 ml	11·25 ≡ 225 µg/m³ using latest conversion factor of 22·5

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0.25	25 ml to 100 ml
0.10	10 ml to 100 ml
0.050	5 ml to 100 ml
0.020	2 ml to 100 ml
0.010	1 ml to 100 ml

These diluted calibration solutions should be prepared fresh every week and stored in the refrigerator.

METHOD

Fill autosampler vials with each of the THBP and scopoletin calibration solutions.

Make duplicate injections of each calibration solution into the combined UVPM/FPM system using the loop injector. Measure the peak area of the UV absorbance peak using an integrator.

Prepare a calibration graph of UV Absorbance Peak Area against UVPM concentration (as calculated from the THBP concentration).

Prepare a calibration graph of Fluorescence Emission Peak Area against FPM Concentration (as calculated from the scopoletin concentration).

Make duplicate injections of the methanol extract of the particulate matter (collected on the Millipore Teflon filter) from its vial into the UVPM/FPM system using the loop injector.

Determine the UV Absorbance Peak Area and the Fluorescence Emission Peak Area.

CALCULATION

By reference to the calibration graphs (or the equation of best fit), determine the UVPM concentration and FPM concentration of the sample solution in ug/ml.

Calculate the quantities of UVPM and FPM per cubic metre of air sampled as follows:

UVPM=U*VM*1000/VA ug/m3

FPM=F*VM*1000/VA ug/m3

Where:

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U=UVPM concentration in methanol extract, in ug/ml

F=FPM concentration in methanol extract, in ug/ml

VM=volume of methanol used to extract the Millipore filter, in ml

VA=volume of air sample collected, in litres

Correct the results for values obtained from the blank.

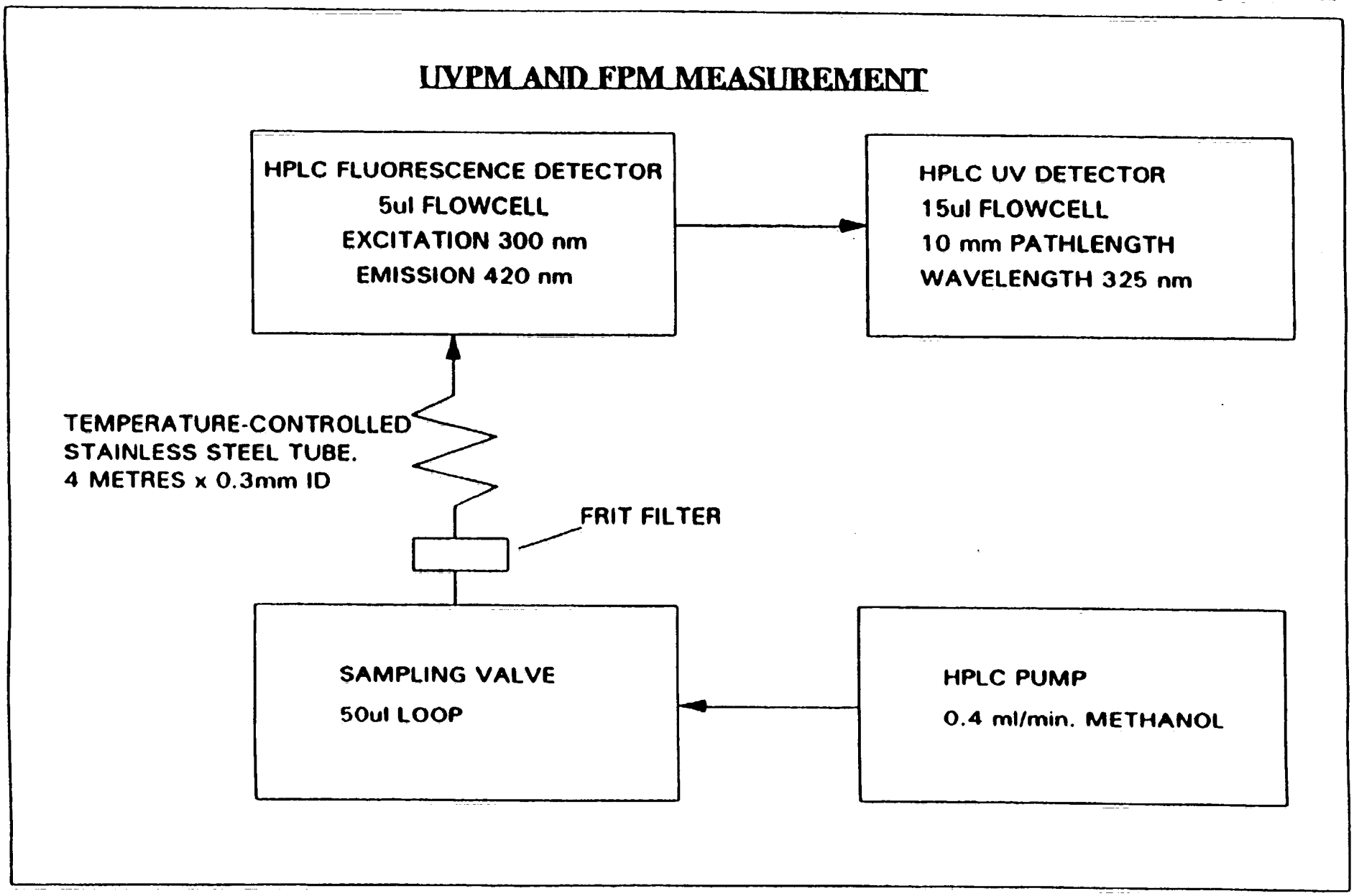
DETECTION LIMITS

The approximate limits of detection for this method are:

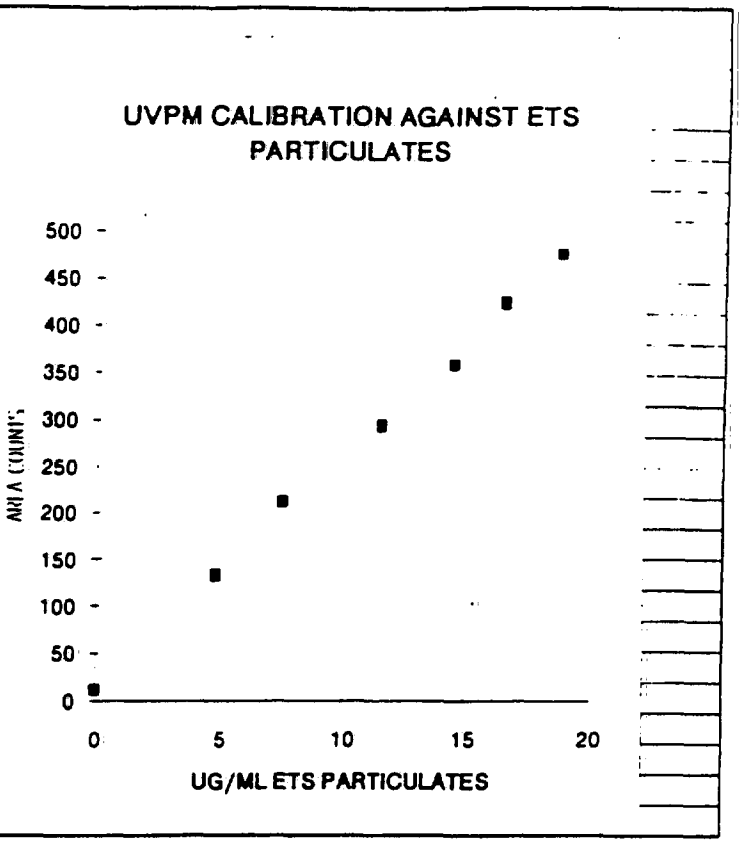
UVPM: 5ug/m3

FPM: 5ug/m3

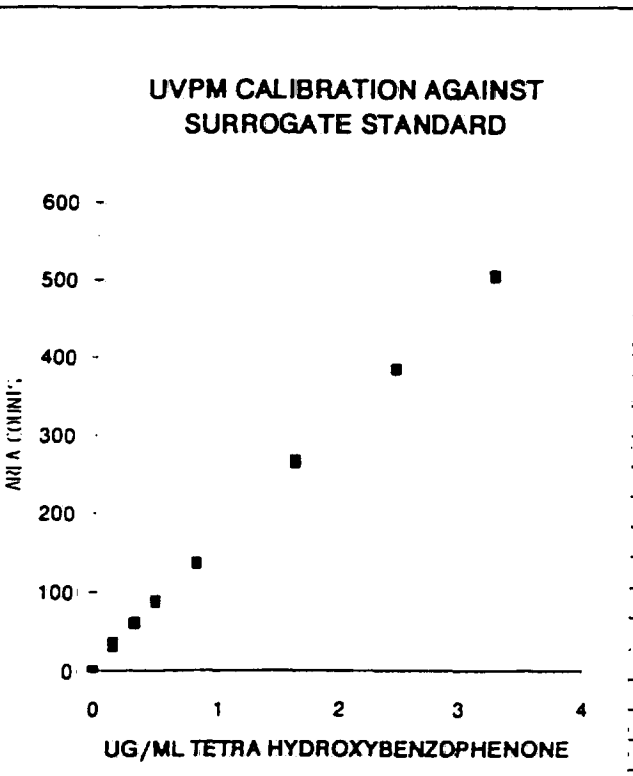
FIGURE 2.



UG/ML	COUNTS
0	10.9
4.75	131.8
4.75	134
7.5	210.4
7.5	212.4
11.5	293.1
11.5	295.3
14.5	356.7
14.5	358.2
16.5	422
16.5	424.5
18.75	474.7

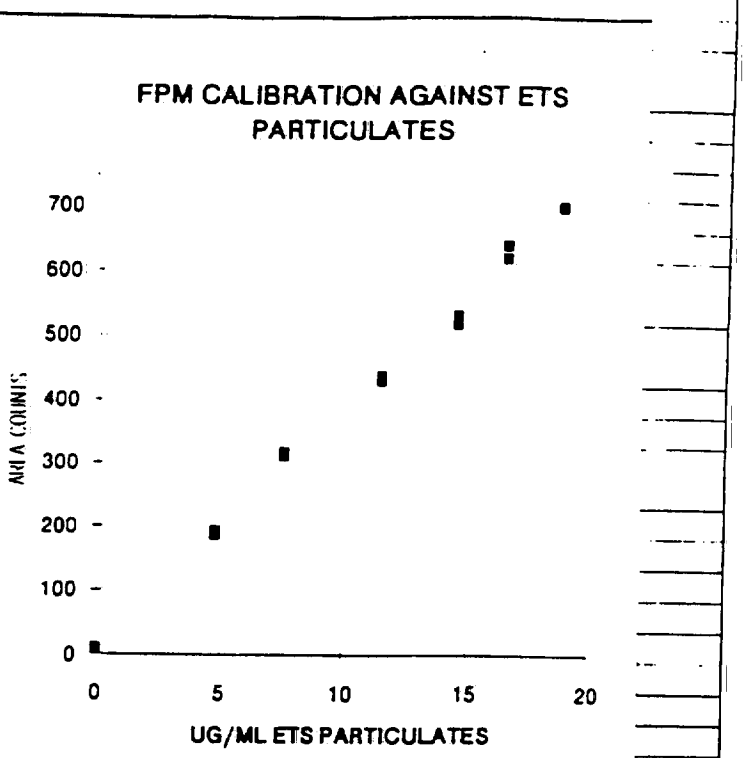


UG/ML	COUNTS
0	0
0.1632	31
0.1632	35.5
0.3264	58.2
0.3264	59.5
0.4896	86.4
0.4896	88.7
0.816	134.5
0.816	135.9
1.632	263
1.632	266.2
2.448	383.5
2.448	384.7
3.264	503.8
3.264	505.6

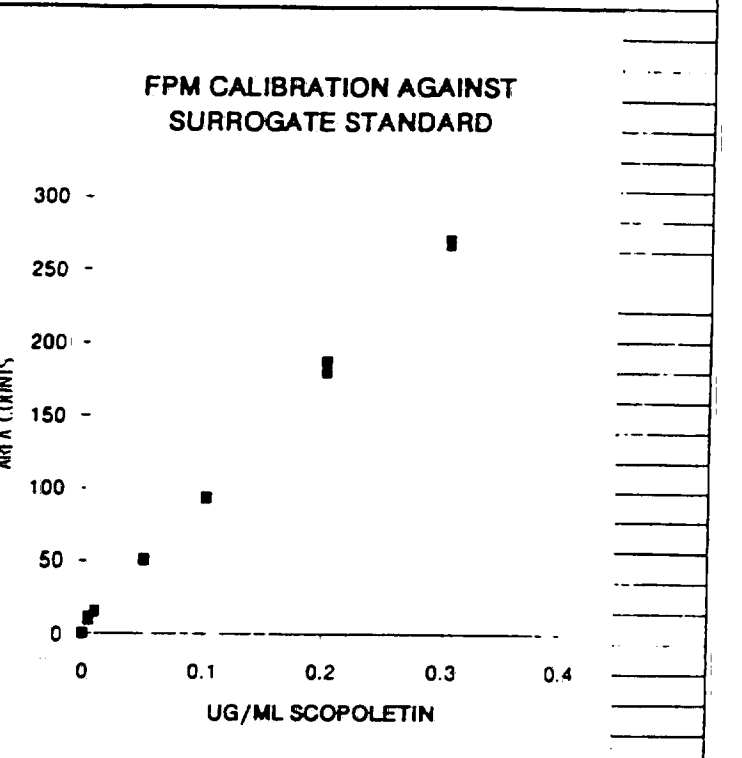


TYPICAL UVPM CALIBRATION RESULTS

UG/ML	COUNTS
0	8.93
4.75	186.9
4.75	191.4
7.5	311.5
7.5	314.7
11.5	429.7
11.5	436.1
14.5	517.3
14.5	530
16.5	620.2
16.5	639.9
18.75	699



UG/ML	COUNTS
0	0
0.00506	8.96
0.00506	11.3
0.01011	14.6
0.01011	15.6
0.05056	50.9
0.05056	50.4
0.10112	92.7
0.10112	93.9
0.20222	179.6
0.20222	186.9
0.30336	266.7
0.30336	270.3



TYPICAL FPM CALIBRATION RESULTS

```
RUN#   327          JUL 30, 1992  14:23:07

AREA%
      RT       AREA TYPE  WIDTH       AREA%
    .630    1333725   BB    .213  100.00000

TOTAL AREA=1333725
MUL FACTOR=1.0000E+00
```

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*  RUN #  328      JUL 30, 1992  14:24:51
START
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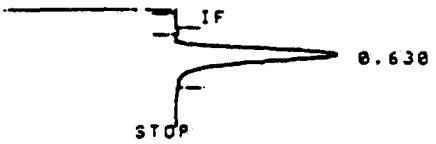

7.50

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RUN#   328          JUL 30, 1992  14:24:51

AREA%
      RT       AREA TYPE  WIDTH       AREA%
    .630    2104210   BB    .213  100.00000

TOTAL AREA=2104210
MUL FACTOR=1.0000E+00
```

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*  RUN #  329      JUL 30, 1992  14:27:03
START
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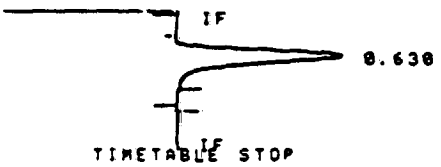


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RUN#   329          JUL 30, 1992  14:27:03

AREA%
      RT       AREA TYPE  WIDTH       AREA%
    .630    2124349   PB    .213  100.00000

TOTAL AREA=2124349
MUL FACTOR=1.0000E+00
```

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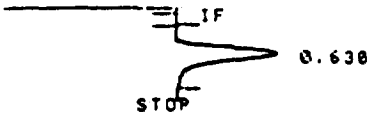
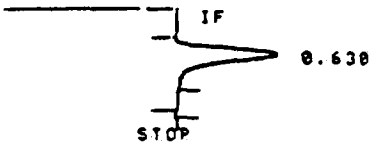
TYPICAL UVPM PEAKS ETS PARTICULATES

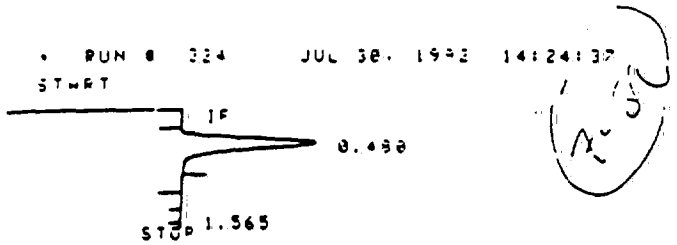
```
RUN#    302              JUL 30, 1992   12:00:58

AREA%
       RT       AREA TYPE  WIDTH       AREA%
     .630     387373   BB    .217   100.00000

TOTAL AREA= 387373
MUL FACTOR=1.0000E+00


*  RUN #  303      JUL 30  1992  12:02:55
START
```




```
RUN#    303              JUL 30, 1992   12:02:55

AREA%
       RT       AREA TYPE  WIDTH       AREA%
     .630    1344627   PB    .218   100.00000

TOTAL AREA=1344627
MUL FACTOR=1.0000E+00


*  RUN #  304      JUL 30, 1992   12:04:58
START
```



TYPICAL UVPM PEAKS SURROGATE STANDARDS

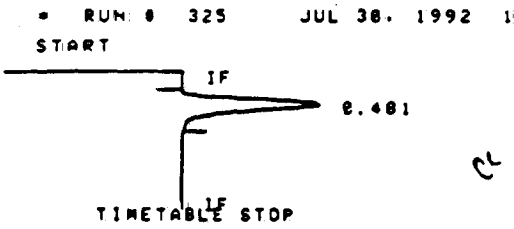


RUN# 324 JUL 30, 1992 14:24:37

AREA%

RT	AREA	TYPE	WIDTH	AREA%
.480	3115869	BB	.185	99.90339
1.565	3013	PP	.114	.09661

TOTAL AREA=3118882
MUL FACTOR=1.0000E+00



RUN# 325 JUL 30, 1992 14:26:49

AREA%

RT	AREA	TYPE	WIDTH	AREA%
.481	3146744	BB	.183	100.00000

TOTAL AREA=3146744
MUL FACTOR=1.0000E+00

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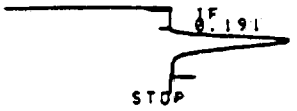
TYPICAL FPM PEAKS ETS PARTICULATES

```
* RUN # 317      JUL 30, 1992  14:10:20
START
```

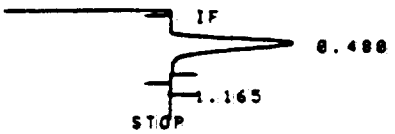



```
RUN#   317          JUL 30, 1992  14:10:20

AREA%
      RT       AREA TYPE  WIDTH      AREA%
    .191       1637   PP   .072     .06134
    .482    2666952   PB   .183   99.93866

TOTAL AREA=2668589
MUL FACTOR=1.0000E+00
```

```
* RUN # 318      JUL 30, 1992  14:12:09
START
```




```
RUN#   318          JUL 30, 1992  14:12:09

AREA%
      RT       AREA TYPE  WIDTH      AREA%
    .488    2783885   BB   .101   99.95552
   1.165       1203   PB   .068     .04449

TOTAL AREA=2784206
MUL FACTOR=1.0000E+00
```

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TYPICAL FPM PEAKS SURROGATE STANDARDS

APPENDIX 2

DETERMINATION OF SOLANESOL CONTENT AND SOLANESOL BASED ETS PARTICULATES (SPM)

PRINCIPLE

Particulate matter collected on a Millipore Teflon filter is extracted with methanol.

The Solanesol content of this extract is measured by HPLC.

Solanesol-based ETS particulate matter is calculated using a pre-determined factor which is calculated from the solanesol content of ETS particlate matter generated in a Model Room.

APPARATUS

HPLC Pump capable of pumping methanol at 2.0ml/min.

UV detector set at 210nm.

Thermostatted column oven set at 30C

Guard column:(Waters) Guard-Pak C18

Analytical column: Such as 25cm Spherisorb ODS 2, 5micron

Loop injector with 200ul loop.

The equipment is set up as shown in FIGURE 3.

REAGENTS

Methanol: (Romil) Super Purity, 99.9% minimum

Solanesol: (Sigma), 90%+. Purity checked against purified solanesol (98%+)

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CALIBRATION

Prepare (by dilution from a stronger solution) a master solution containing 2.5mg of solanesol in 2.5 litres of methanol(ie 1ug/ml).

Store this solution in a 2.5 litre amber Winchester reagent bottle used previously to store methanol.

This solution is stable for at least six months when stored in a refrigerator.

Prepare calibration solutions by dilution of the master solution with methanol as follows:

CONCENTRATION ug/ml	DILUTION OF MASTER	EQUIVALENT SPM ug/ml
0.5	50ml to 100ml	
0.25	25ml to 100ml	
0.10	10ml to 100ml	
0.050	5ml to 100ml	
0.020	2ml to 100ml	
0.010	1ml to 100ml	

These diluted calibration solutions should be prepared fresh every week and stored in the refrigerator.

NOTE: Allowance must be made for the purity of the solanesol in calculating the strength of these calibration solutions

METHOD

Fill an autosampler vial with each of the solanesol calibration solutions.

Make duplicate injections of each calibration solution into the HPLC system using the loop injector.

Allow the separation to continue to 1.5 times the solanesol retention time in order to elute later peaks.

Measure the peak area of the UV absorbance peak corresponding to solanesol using an integrator. For the lower level calibrations

2023703525

manual peak height measurements are more reliable than integrator peak areas.

Prepare a calibration graph of Peak Area against solanesol concentration.

Make duplicate injections of the methanol extract of the particulate matter (collected on the Millipore Teflon filter) from its vial into the HPLC system using the loop injector.

Determine the Peak Area/Peak Height corresponding to solanesol using an integrator/ruler.

CALCULATION

By reference to the calibration graph (or the equation of best fit), determine the solanesol concentration of the sample solution in ug/ml.

Calculate the quantity of solanesol per cubic metre of air sampled as follows:

Solanesol=S*VM*1000/VA ug/m3

Calculate the quantity of SPM per cubic metre of air sampled as follows:

SPM=Solanesol*FS ug/m3

Where:

S=solanesol concentration in methanol extract, in ug/ml

VM=volume of methanol used to extract the Millipore filter, in ml

VA=volume of air sample collected, in litres.

FS=Factor to convert solanesol to SPM Current factor = 3.85 for UK cigarettes.

Correct the results for values obtained from the blank.

DETECTION LIMIT

The approximate limit of detection for this method is 10ug/m3 of SPM

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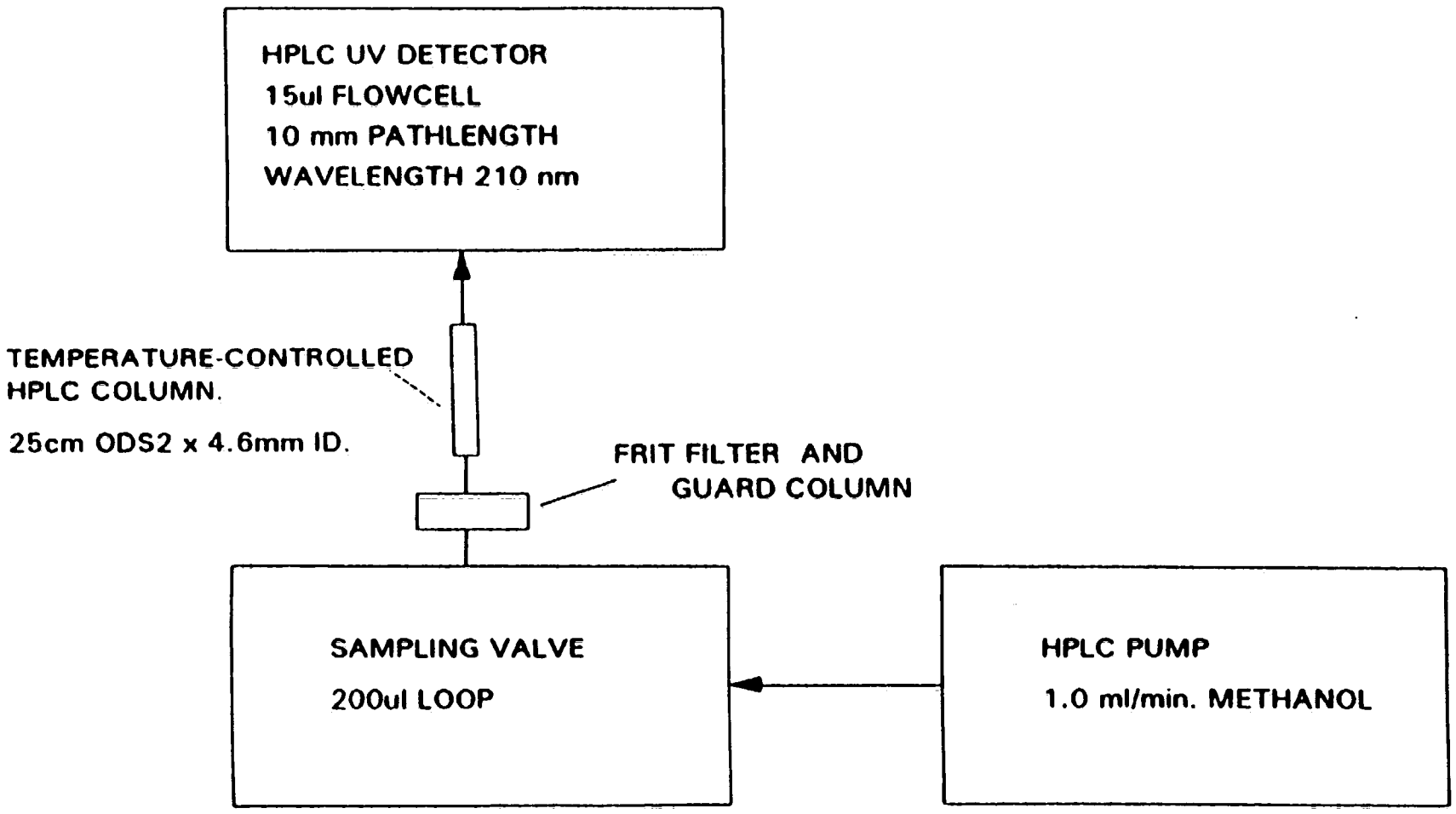
FIGURE 3

SOLANESOL MEASUREMENT



56.

PHILLIPS

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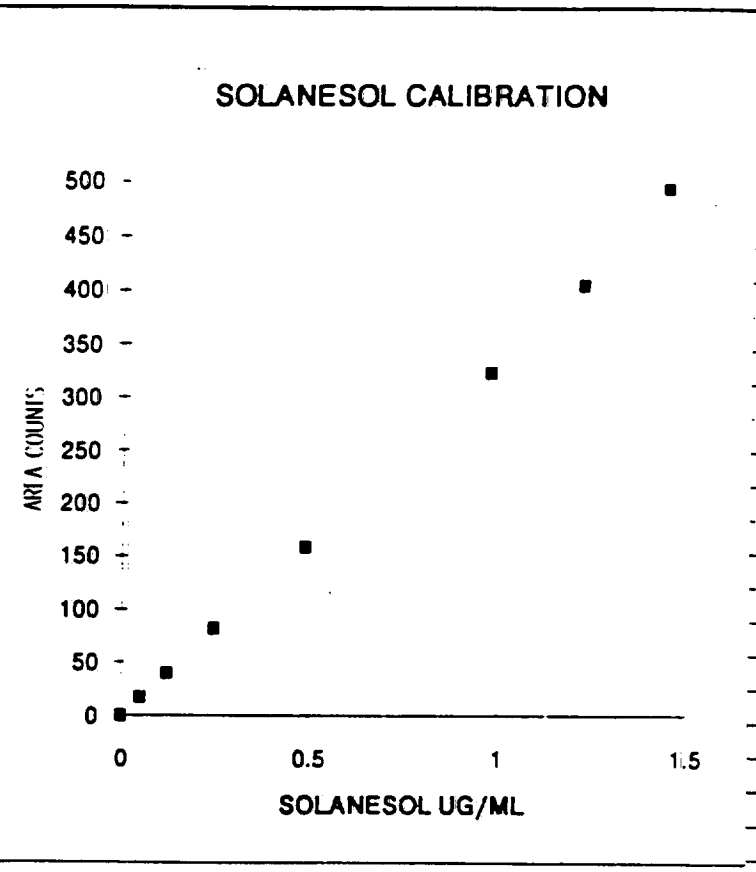
UG/ML	COUNTS
0	0
0.0488	17
0.122	39.8
0.244	81.6
0.488	158.2
0.976	323
1.22	404.2
1.44	493.3



TYPICAL SOLANESOL CALIBRATION RESULTS

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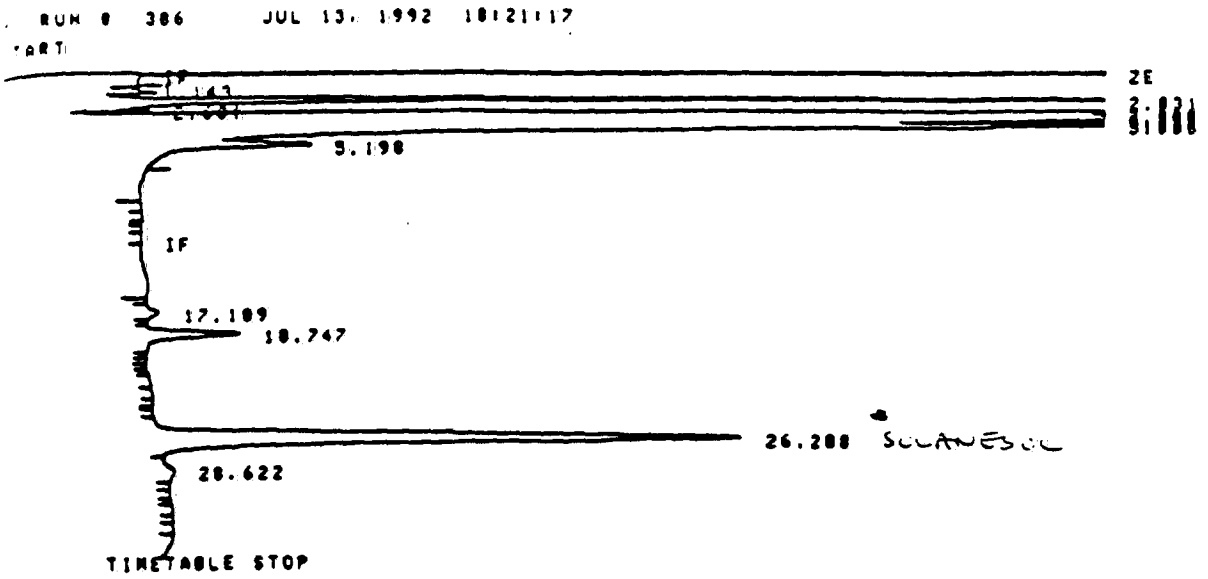


RUN# 386 JUL 13, 1992 18:21:17

SAMPLE# 4

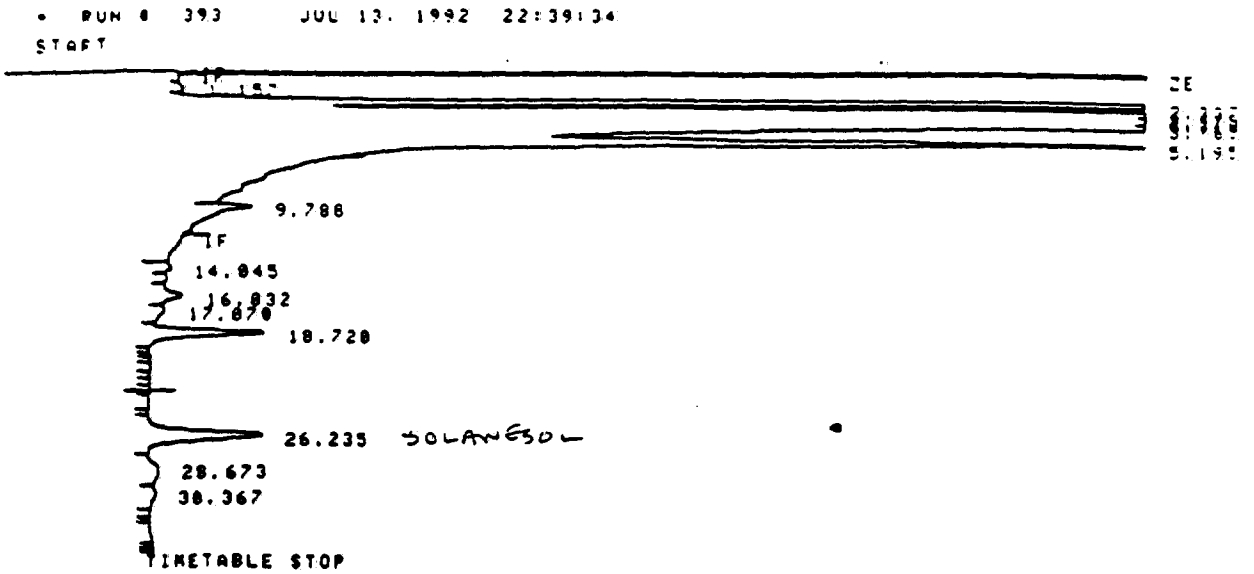
AREA%

RT	AREA	TYPE	WIDTH	AREA%
1.143	2861	BB	.110	.01708
2.831	843385	VV	.219	5.03501
2.681	36979	VP	.160	.22076
3.888	3283517	PV	.204	19.60261
3.327	7560624	VV	.265	45.13696
3.888	2750277	VV	.490	16.41916
5.198	468686	VB	.650	2.79758
17.189	26586	PB	.527	.15872
18.747	176353	VB	.500	1.05283
26.288	1576872	PB	.714	9.41393
28.622	24351	BB	.675	.14538

TOTAL AREA=1.6758E+07
MUL FACTOR=1.0000E+00

TYPICAL SOLANESOL CHROMATOGRAM:STANDARD

2023703529



RUN# 393 JUL 13, 1992 22:39:34

SAMPLE# 11

AREA%

RT	AREA	TYPE	WIDTH	AREA%
1.157	7977	VP	.308	.03347
2.337	4509242	PV	.224	18.92826
2.973	2652418	VV	.317	11.12922
3.318	9871558	VV	.292	41.41995
3.765	4276835	VV	.422	17.94176
5.195	1802676	VB	.513	7.56383
9.788	52385	BB	.418	.21988
14.845	9878	BB	.363	.04145
16.832	42983	VB	.526	.18035
17.870	15111	BB	.607	.06340
18.728	218614	BB	.511	.91728
26.235	318286	PB	.736	1.33549
28.673	40593	BB	1.081	.17032
38.367	15134	BB	.746	.06350

TOTAL AREA=2.3833E+07
MUL FACTOR=1.0000E+00

TYPICAL SOLANESOL CHROMATOGRAM.ETS

APPENDIX 3

DETERMINATION OF NICOTINE

PRINCIPLE

Nicotine collected on the front filter (Millipore, Teflon) is extracted into methanol. An aliquot of this extract is basified with sodium hydroxide and the nicotine is extracted into DIPE which contains triethylamine and an internal standard.

The nicotine collected on the second, acidified, pad is basified with sodium hydroxide and the nicotine is extracted into DIPE which contains triethylamine and an internal standard.

APPARATUS

GC, on-column injector, NPD, integrator.

Typical GC conditions are:

Column: 10m * 0.53mm CPSIL5 CB (Chrompack)

Carrier gas: Helium 5ml/min.

Initial temperature: 40C

Hold time: 0.5 min.

Temperature programme: 15C/min.

Final temperature: 165C

Final time: 5 min.

Injection volume: 1.0ul

REAGENTS

Di-isopropyl ether (DIPE): (Romil) Super Purity, 99.9% minimum

DIPE solvent. Containing (1) 0.1 ml/litre triethylamine

(2) 2.0 mg/litre N-ethylnornicotine.

Nicotine: Double distilled 99%+

N-ethyl nornicotine: Double distilled 99%+

CALIBRATION

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Prepare (by dilution from a stronger solution) a master solution containing 25mg of nicotine in 2.5 litres of DIPE solvent (ie 10 ug/ml).

NOTE: The DIPE solvent contains 0.25 ml of triethylamine and 5mg of N-ethylnornicotine internal standard. All dilutions from this master solution are made with the same solvent mixture.

Store this solution in a 2.5 litre amber Winchester reagent bottle used previously to store methanol.

This solution is stable for at least six months when stored in a refrigerator.

Prepare calibration solutions by dilution of the master solution with DIPE solvent as follows:

CONCENTRATION ug/ml	DILUTION OF MASTER
5.0	50ml to 100ml
2.5	20ml to 100ml
1.0	10ml to 100ml
0.50	5ml to 100ml
0.25	2ml to 100ml
0.10	1ml to 100ml

These diluted calibration solutions should be prepared fresh every week and stored in the refrigerator.

METHOD

Fill an autosampler vial with each of the nicotine calibration solutions.

Make duplicate injections of each calibration solution into the GC system. Measure the area of the NPD peaks corresponding to nicotine and the internal standard using an integrator.Prepare a calibration graph of the ratio of nicotine peak area to internal standard peak area against nicotine concentration.

Make duplicate injections of the nicotine extract to be tested from its vial into the GC.

Determine the peak areas corresponding to nicotine and internal standard using an integrator and calcuate the ratio of nicotine peak area to internal standard peak area.

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CALCULATION

By reference to the calibration graph (or the equation of best fit), determine the nicotine concentration of the sample solution in ug/ml.

Calculate the quantity of nicotine per cubic metre of air sampled as follows:

Nicotine =N*VM*1000/VA ug/m3 for the first filter

Nicotine =N*1000/VA ug/m3 for the second filter

Where:

N=the nicotine concentration, in ug/ml

VA=volume of air sample collected, in litres

Add the values for the first and second filters to calculate the total nicotine concentration.

Correct the results for values obtained from the blank.

DETECTION LIMITS

Approximate detection limits for this method are:

Front filter: 2ug/m3

Acidic filter: 0.5ug/m3

NICOTINE EXTRACTION EFFICIENCY

Nicotine concentration	Extraction into DIPE from 5N NaOH (1)	Extraction into DIPE from 5N NaOH+methanol(2)
1.21	1.23 (101.5%)	1.14 (94.7%)
0.60	0.621 (103%)	0.569 (94.2%)

All results in ug/ml

1. Extraction from 4ml 5N NaOH into 1ml DIPE

2. Extraction from 4ml 5N NaOH and 1ml methanol into 1 ml DIPE

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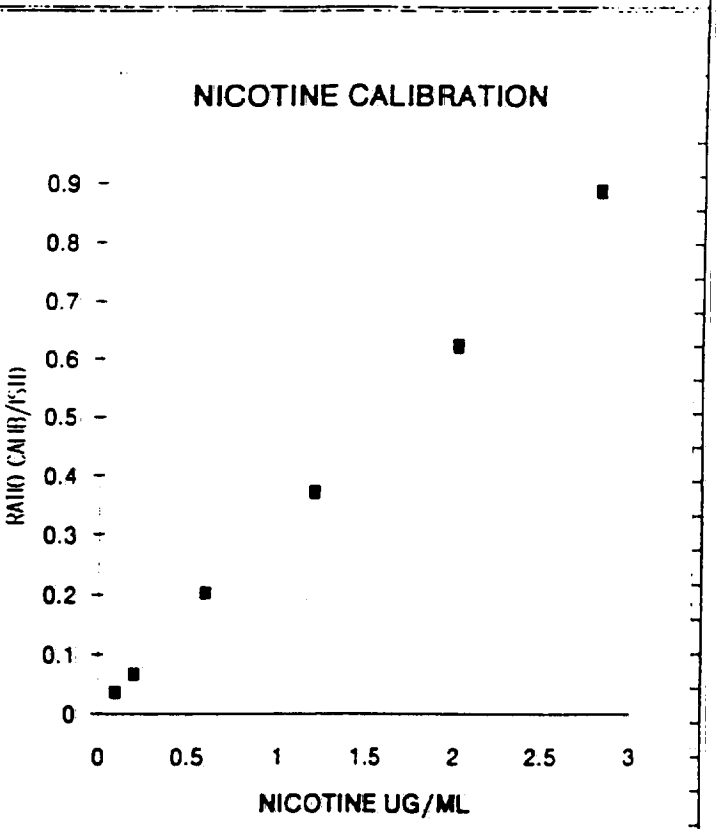
UG/ML	RATIO C/ISTD
0.1006	0.03648
0.1006	0.03539
0.2012	0.06768
0.2012	0.06672
0.6036	0.20381
0.6036	0.20283
1.2072	0.37075
1.2072	0.37297
2.012	0.61819
2.012	0.623
2.8168	0.8821
2.8168	0.88655



TYPICAL NICOTINE CALIBRATION RESULTS

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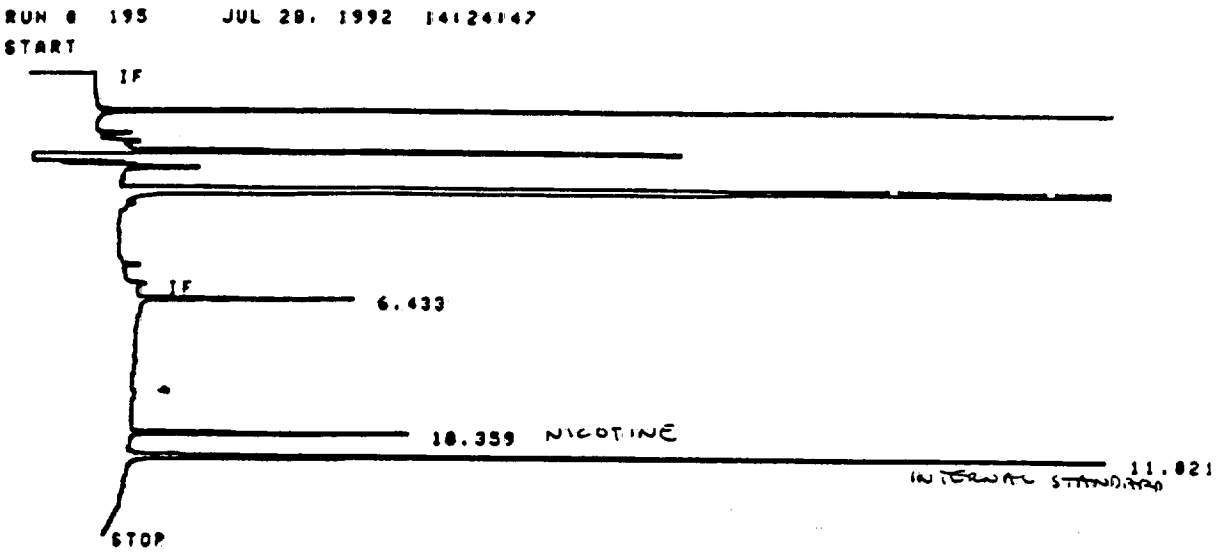

RUN# 195 JUL 28, 1992 14:24:47

SAMPLE# 14

AREA%

RT	AREA	TYPE	WIDTH	AREA%
6.433	18825	VB	.853	10.31965
18.359	15444	BB	.858	14.72382
11.821	78628	PB	.859	74.95734

TOTAL AREA= 184897
MUL FACTOR=1.0000E+00

TYPICAL NICOTINE SEPARATION: CALIBRATION

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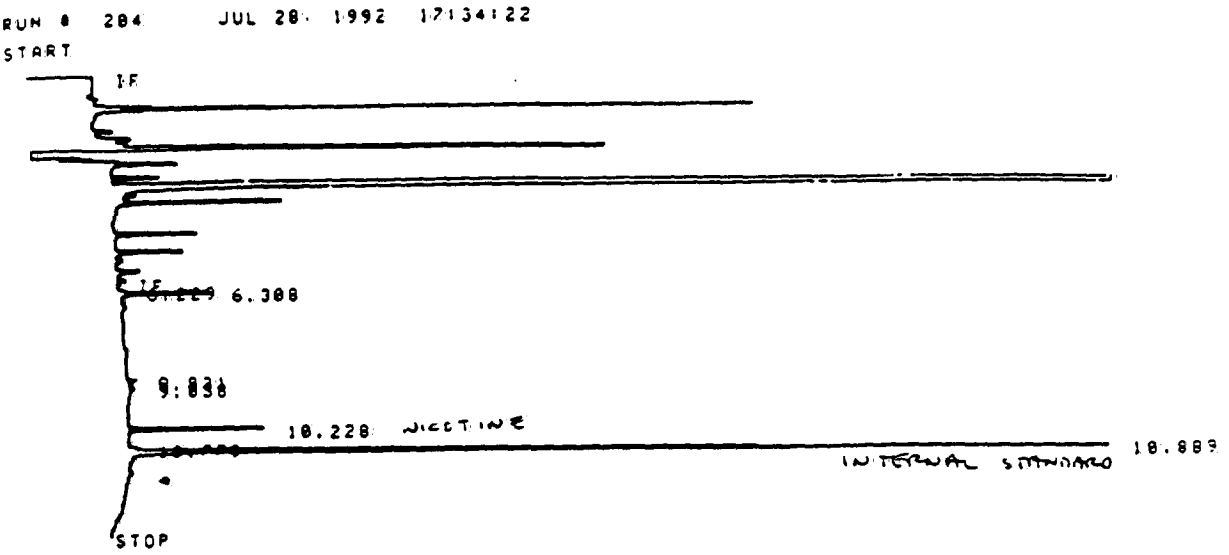

RUN# 284 JUL 28, 1992 17:34:22

SAMPLE# 23

AREA%

RT	AREA	TYPE	WIDTH	AREA%
6.229	419	VV	.056	.50450
6.388	5189	VV	.060	6.24789
8.831	639	BV	.069	.76940
9.838	898	VV	.104	1.08125
10.228	7863	PB	.060	9.46756
10.778	278	PV	.043	.33473
10.889	67766	VB	.060	81.59466

TOTAL AREA= 83052
MUL FACTOR=1.0000E+00

TYPICAL NICOTINE SEPARATION: ETS SAMPLE

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APPENDIX 4

SALIVARY COTININES: SAMPLE COLLECTION AND ANALYSIS

SALIVETTE INSTRUCTIONS FOR USE

COLLECTION OF SALIVA

1. The cylindrical shaped swab (b) is removed from the insert (c) and placed in the mouth.

2. The swab is chewed for 30 to 45 seconds or until one can no longer prevent swallowing the saliva produced.

   If the swab can not be chewed, it can be placed under the tongue for 30 to 45 seconds.

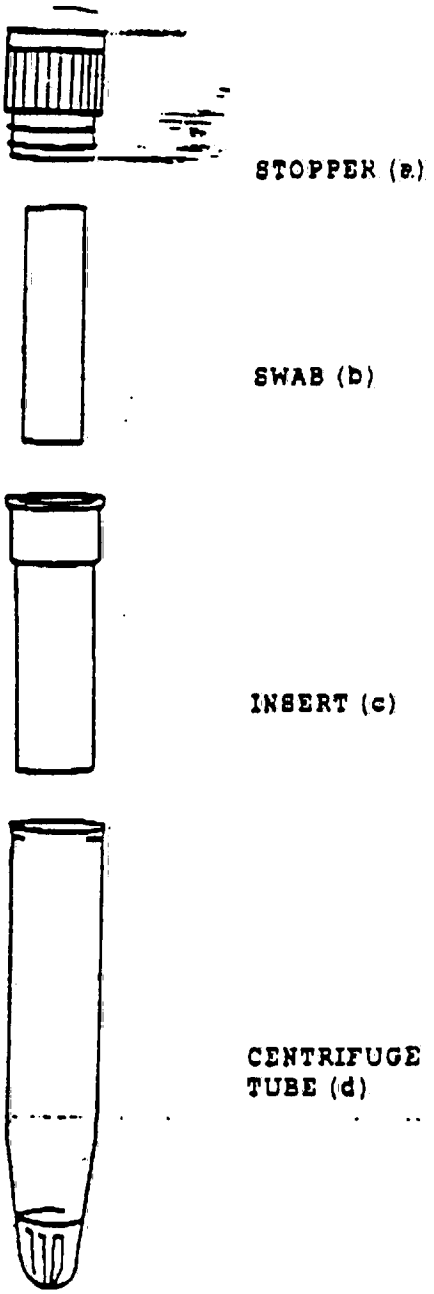
3. After the above procedure is complete, the swab is returned to the insert and the Salivette firmly closed with the stopper (a).

   Storage conditions are dependent upon specimen use and have not been established.

RECOVERY OF SALIVA

4. The Salivette is centrifuged for two minutes at 1000 x G. Higher G forces result in only slightly higher yields of saliva.

   During centrifugation, the saliva will pass from the cylindrical shaped swab through the hole in the bottom of the suspended tube into the clear centrifuge tube. Mucous strands and particles will be caught in the conical tip of the centrifuge tube allowing easy decanting of the clear saliva.



STOPPER (a)

SWAB (b)

INSERT (c)

CENTRIFUGE TUBE (d)

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SARSTEDT

YOU HAVE BEEN ASKED TO PROVIDE A SALIVA SAMPLE: IT'S EASY! CAREFULLY READ AND FOLLOW THE STEPS BELOW.

1. Hold the vial in an upright position (cap at the top).

2. Remove the cap and hold the vial to your lips.
(Do not touch the cotton pad with your fingers!)

3. Tilt the vial so that the cotton pad slides into your mouth.

4. Chew the cotton pad vigorously for a minute or two until the pad is completely saturated.

5. Place the vial to your lips and allow the cotton pad to slide back into the vial. DO NOT SPIT INTO THE VIAL and DO NOT USE YOUR FINGERS. If you need to, use your tongue to guide the pad back into the vial.

6. Put the cap back on the vial.

2023703540

RESERCA
MEMBER OF SWEDISH TOBACCO

1991-09-09

ANALYTICAL METHOD FOR NICOTINE AND COTININE IN SALIVA

SUMMARY: Nicotine and cotinine are extracted from alkalized saliva by use of methylene chloride. The two components are quantified by use of gas chromatography using a N-selective detector. Structurally similar analogues are used for internal standards.

EQUIPMENT:

GC system: VARIAN model 3700, 3400, 3500, equipped with splitless injectors and nitrogen sensitive detectors.

Capillary columns: SP-1000 (Nanocoat, Finland) 25 m x 0.32 mm i.d. 0.25 um coating.

Integrators: Shimadzu C-R3A, C-R5A and HP 3388A

Sample concentrators: Pierce Reacti-Therm

Centrifuge: Heraeus Christ cooled model Minifuge T, equipped with rotor 1B. Radius 18.4 cm.

Shaker: Desaga Shaker

Test tubes: 5 ml Becton Dickinson Vacutainer tubes with 0.5 ml citrate solution, equipped with Teflon septum in screw caps (Labora).
10 ml centrifuge tubes with Teflon septum in screw caps.

Sample vials: 2 ml septum vials (VARIAN) with conical bottom (made in the lab)
2 ml brown septum vials

Pipettes: 5-1000 ul Automatic dispensing pipettes model Pipetman (Gilson) and Transferpette (Brand). Pasteur pipettes.

GC syringes: 1.0 ul Hamilton 7001.

Reagents: Sodium hydroxide (Pronalys, M&B). 5 M solution shaken with methylene chloride before use.
Methylene chloride (UV spectroscopy quality, Fluka AG)
Ethanol 99.9% (Spectroscopy quality, Kemetyl)

2023703541

STANDARDS AND CALIBRATION SOLUTIONS:

(-)-Nicotine: Vacuum distilled and stored under nitrogen in brown ampoules at -80°C Standards are made by weighing 100 mg nicotine into a 100 ml measuring glass (brown) and dissolving in EtOH. This stock solution is then diluted 1:10, 1:100 and 1:1000.

(-)-Cotinine: Our own synthesis according to McKennis. Double distilled under vacuum. Chrystals are stored in a dark container at -20°C. Stock solution and dilution series as for nicotine.

Internal standards: *N-methylanabasine* (for the quantification of nicotine) and *N-ethylnorcotinine* (for the quantification of cotinine) are syntizised according to McKennis and purified by flash and column chromatography.
Stock solutions are prepared as for nicotine and cotinine. From the stock solutions are prepared combined internal standard solutions, the concentrations depending on level of analyts expected.

Calibration solutions: 1:1 solution containing equal amounts of nicotine, cotinine, methylanabasine and ethylnorcotinine. 0.5 ml of solutions having the concentration 1.0 mg/ml are dispensed into a 100 ml measuring glass and diluted to volume with EtOH. This gives 5 ug/ml of each analyte.The solution is kept in brown septum vials at -20°C.
For low concentrations this calibration solution is diluted 1:10.

SAMPLE PREPARATIONS:

Saliva is collected in 5 or 10 ml test tubes and is stored at -20°C.

WORK UP OF SAMPLES:

The frozen saliva samples are thawed and centrifuged for 10 minutes at 3000 rpm(1850 g) and at a temperature of 10°C.
0.5-1-0 ml clear supernatant is collected and to that is added internal standard solutions. Normally 200 ng of methylanabasine and ethylnorcotinine is acceptable level for work with smokers. To the sample is added 1ml 5M NaOH and 2 ml methylene chloride.
The sample is shaken for 5 minutes and then centrifuged for 20 minutes at 3000 rpm and a temperature of -5°C.
The supernatant water phase is removed with a Pasteur pipette connected to a water ejector. The organic layer is decanted into sample vials that are stored at -20°C until used for analysis. The content in the vial is then concentrated to approximately half the volume and then EtOH is added and the content is further concentrated down to 10-50 ul.

GC PARAMETERS:

Carrier gas: Helium. Flow rate 2.4 ml/min
Detector gas:H_2. Flow rate 3.3 ml/min. Make up He at 27 ml/min. Air at 240 ml/min.
Injector temperature: 290 °C

Detector temperature: 300 °C
Injection volume: 0.1-0.3 ul

Oven temperature: Initial temp 135 °C. Initial time 10 minutes
Temp programming rate 30 degrees per minute
Final temp 220 oC. Final time 15 min.

Retention times: Nicotine 5.1 minutes. Methylanabasine 7.7 minutes
Cotinine 16.9 minutes. Ethylnorcotinine 17.3 minutes

CALCULATIONS:

Relevant retention times, response factors and amounts of internal standards are programmed into the integrator for calculation of amounts of analyte.
Response factors are found by dividing the area for the Internal Standard by the area for the analyte.

VALIDATION:

Calibration: The GC performance is evaluated by injecting Calibration Solution and responce factors and retention times are controlled in the beginning and at the end of each series of samples and furthermore at least once a month.
The total method is checked by using samples of saliva pooled from a number of tobacco users.

Liniarity: The liniarity of the GC responce has been validated by using standard solution and constructing a standard graph. Saliva from non-tobacco users (0-saliva) is spiked with nicotine in concentrations 1-10 ng/ml and cotinine 1-10 ng/ ml and 5-500 ng/ml.

Sensitivity:

Detection limits are 5 pg for both nicotine and cotinine at a S/N ratio of 2.
Minimum detectable concentration is 0.5 ng/ml

Reproducibility:

The reproducibility has been verified by the use of standard solution graphs.
Coefficients of variation for both variability during the same day and during different days are given in the table on next page.

Yield of extraction:Nicotine and cotinine can be quantitatively extracted from saliva by the used method.

2023703543

PRECISION OF DETERMINATION OF NICOTINE AND COTININE IN BODY FLUIDS

Nicotine	Coefficients of variation (%)			
Amount added (ng/ml)	Plasma (n=6)	Saliva (n=6)	Breast milk (n=6)	Urine (n=6)
1	8.5	12.3		18.1
2	9.1	8.3		7.0
5	6.9	4.9	4.5	10.9
10	4.3	7.2	5.5	9.6
50	2.7	2.0	3.9	3.4
100	3.6	3.0	1.2	4.4

Cotinine	Coefficients of variation (%)			
Amount added (ng/ml)	Plasma (n=6)	Saliva (n=6)	Breast milk (n=6)	Urine (n=6)
1	8.8	12.0		19.5
2	6.9	11.6		9.5
5	8.5	11.0	5.7	10.6
10	1.6	3.1	5.1	6.4
50	2.3	4.9	5.4	3.9
100	1.5	3.9	1.5	2.8

2023703544

PE11

HAZLETON UK

CURRICULUM VITAE

1. PERSONAL DETAILS

Name: HOUSEMAN, Terence Henry

Date of Birth: REDACTED

Job Title: Head of Business Development, Chemical and Medical Sciences

Education:

REDACTED University of Bradford
1st class Honours Bachelor of Technology Degree in Industrial Chemistry (specialising in high polymer chemistry and chemistry of dye stuffs).

REDACTED University of London
PhD thesis entitled "Radiochemical studies of the oxidation of natural rubber".

2. PRESENT EMPLOYMENT

Hazleton UK
REDACTED Head of Business Development, Chemical and Medical Sciences.
Responsible for a complete commercial/business support function which embraces and oversees all financial budgeting, planning and business development activities of the division.

a CORNING Laboratory Services Company

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REDACTED	Director of Chemical and Medical Sciences Responsible to the Managing Director for all scientific and commercial aspects of the Division, which now incorporates Hazleton's clinical activities.
REDACTED	Director of Chemistry, Responsible to the Managing Director for all aspects of the administration, organisation and management of assigned operating areas, including Analytical Chemistry, Metabolism and Pharmacokinetics and HAZLETON Masspec (and until recently, Central Dispensary). Also responsible for the commercial activity of the Division.
REDACTED	Head of Chemistry and Metabolism Responsible to the Director of Research Operations for all operational and scientific aspects of Analytical Chemistry, metabolism and Pharmacokinetics and Central Dispensary, including establishment of the latter. This also included delegated responsibility for commercial activities such as business development.
REDACTED	Head of Radio and Analytical Chemistry Responsible for establishing and developing the department as a viable profit centre, with responsibility for all operational and scientific aspects and delegated responsibility for business development.

3. PAST EMPLOYMENT

REDACTED	Tobacco Research Council Laboratories Head of Chemistry Research Services (and Radiological Protection Officer)

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Responsible for providing a chemistry service (analytical and synthetic) to other departments within the company, eg Pharmacology, Biology, and collaborating with external TRC grantees.

REDACTED

Tobacco Research Council Laboratories
Radioanalytical Chemist
Responsible for studying the smoke transference of exogenous and endogenous tobacco constituents to mainstream and substream smoke with the ultimate objective of determining the fate of constituents in the human smoking situation.

REDACTED

Natural Rubber Producers' Research Association
Radioanalytical Chemist
Responsible for studying mechanisms of oxidative mainstream and cross-link scissions in vulcanized and unvulcanized natural rubber. Radioanalytical techniques featured strongly in this work.

REDACTED

Undergraduate
Associated Chemical Companies Ltd, Central Research Laboratories
Industrial training period

REDACTED

Sandoz Products (Dye Stuffs) Ltd
Industrial training period

REDACTED

Undergraduate
Natural Rubber Producers' Research Association
Industrial training period

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4. TRAINING COURSES

1972	Radiological Protection Course - Harwell
1980	HLE Senior Management residential training course Plus numerous specialist short courses on accountancy, management, etc.

REDACTED

6. SCIENTIFIC PUBLICATIONS

Barnard, D, Houseman, T H, Porter, M R and Tidd, B K (1969) "Thermal racemization and cis, trans - isomerization of allylically unsaturated di - and poly-sulphides: a mechanism involving branched sulphur chains".
Chem. Comm. - 371

Barnard, D and Houseman, T H (November 1969) "The application of radiochemical methods to the study of oxidation of natural rubber". Symposium of Oxidation, Hall of Fame Ceremony, Institute of Polymer Science. Adron University.

Ayrey, G, Barnard, D and Houseman, T H (1971) "The use of radioisotopically labelled analytical reagents in organic chemistry".
Chem Rev 71 371

Barnard, D, Cain, M E, Cuneen, J I and Houseman, T H (1972) "Oxidation of vulcanized natural rubber".
Rubber Chem Technol, 45, 381

NB This paper was also presented at a meeting of the Division of Rubber Chemistry, American Chemical Society, Cleveland, Ohio, October 1971.

Ayrey, G, Barnard, D and Houseman, T H (1974) "The synthesis of tritium labelled dialkenyl sulphides structurally related to sulphur crosslinks in vulcanized natural rubber".
J Labelled Compound, 10, (1), 121

Houseman, T H and Heneage, E (1973)
"Studies of cigarette smoke transfer using radioisotopically labelled tobacco constituents. Part I - The preparation of radioisotopically labelled cigarettes".
Beitrage Zur Tobakforschung, Z, (3), 138

Houseman, T H (1973)
"Studies of cigarette smoke transfer using radioisotopically labelled tobacco constituents. Part II - The transference of radioisotopically labelled nicotine to cigarette smoke".
ibid. Z, (3), 142

NB This paper was also presented at the 25th Tobacco Chemists Res Conf, Louisville, Kentucky, USA, 1971

Davis, B R, Houseman, T H and Roderick, H R (1973) "Studies of cigarette smoke transfer using radioisotopically labelled tobacco constituents. Part III - The use of dotriacontane-16, 17-^{14}C as a marker for the deposition of cigarette smoke in the respiratory system of experimental animals".

Armitage, A K, Houseman, T H, Turner, D M and Wilson, D A (1974) "The evaluation of a machine for introducing tobacco smoke into the lungs of anaesthetized animals during spontaneous respiration".
Quart J Exp Physiol, 59, 43

Armitage, A K, Houseman, T H and Turner, D M (1974)
"The transfer of endogenous and exogenous radioisotopically labelled nicotine to mainstream cigarette smoke and its absorption into the blood of anaesthetized cats".
ibid, 59, 55

Hopper, J B and Houseman, T H (1974)
"The transference of endogenous and radioisotopically labelled exogenous nicotine to cigar smoke".
Tobacco Science, 18, 160
NB This paper was also presented at the 27th Tobacco Chemists Res Conf, Winston-Salem, North Carolina, October 1973.

Armitage, A K, Dollery, C T, George, C F, Houseman, T H, Lewis P J and Turner, D M.
"Absorption and metabolism of nicotine by man during cigarette smoking".
Brit J Clin Pharmac

Armitage, A K, Dollery, C T, George, C F, Houseman, T H, Lewis, P J and Turner, D M (1975) "Absorption and metabolism of nicotine from cigarettes".
Br med J, 4, 313

Armitage, A K, Dollery, C T, Houseman, T H, Kohner, E M, Lewis, P J and Turner, D M (1977)
"Absorption of nicotine by man during cigar smoking".

Houseman, T H, Macfarlane, E A, Pullinger, D H, and Simons P J (1977)
"A single animal smoking system for exposing rats and other rodents to cigarette smoke".
J Aerosol Sci, 8, 111

Houseman, T H and Pullinger, D H
"Dosimetry of cigarette smoke (and other aerosols) in laboratory animals".
Clinical Toxicology, Proceedings of the European Society of Toxicology, Volume 18, ICS No 417, Amsterdam - Oxford, Exorpa Medica, 1977, pp 265 - 266

Binns, S H, Houseman T H and Phillips, K (1978)
"Synthesis of High specific activity tritium - labelled dotriacontane P
J Labelled Compounds and Radiopharmaceuticals, 14, (2), 163

Armitage, A K, Dollery, C T, Houseman, T H, Kohner, E M, Lewis, P J and Turner, D M (1978)
"The absorption and metabolism of nicotine from cigars".
Clin Pharmcol Ther, 23, (2), 143

7. SCIENTIFIC PRESENTATIONS

Barnard, D, and Houseman, T H (November 1969) "The application of radiochemical methods to the study of oxidation of natural rubber". Symposium on Oxidation, Hall of Fame Ceremony, Institute of Polymer Science. Akron University, November 1969.

Houseman, T H and Heneage, E (1973)
"Studies of cigarette smoke transfer using radioisotopically labelled tobacco constituents. Part I - The preparation of radioisotopically labelled cigarettes".

Hopper, J B and Houseman, T H (1974)
"The transference of endogenous and radioisotopically labelled exogenous nicotine to cigar smoke".

8. OTHER DETAILS

Chartered Chemist; Honorary Representative of the Royal Society of Chemistry; Past Member of the Committee of the Central Yorkshire Section of the Royal Society of Chemistry and the programme sub-committee; Member of HLE Management Group.

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PHILLIPS

PE11

HAZLETON UK

CURRICULUM VITAE

1. PERSONAL DETAILS

Name: FREEMAN, John Michael Howard

Date of Birth: REDACTED

Job Title: Senior Study Director

Education:
1958-1964 Reade Grammar School, Drax
1965-1967 Kitson College, Leeds
1967-1970 Leeds Polytechnic
1970-1974 Sheffield Polytechnic

'O' Levels in English Language, English Literature, Art, French, History, Physics, Geography, Mathematics, Agricultural Science, Physics with Chemistry, Physics.
ONC in Sciences 1967
HNC in Chemistry 1970
BSc Hons. in Applied Chemistry (2:2) 1974
Chartered Chemist (C Chem)
Member of the Royal Society of Chemistry (MRSC)

2. PRESENT EMPLOYMENT

Hazleton UK

REDACTED Senior Study Director, AgVet Residues.
Acts as the focal point of Study Control and has overall responsibility for the scientific and technical

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Dow Chemical Co., Letcombe Regis, Oxfordshire.
Graduate Chemist.
Working in the Residue Environmental Metabolism Group responsibilities included supervision of students during industrial training, developing methods of analysis, liaising with various groups within the Company, producing reports necessary for Registration Authorities, organisation and supervision of "one off" type residue studies, eg operator Monitoring Studies. They also include the implementation of the Zymark Laboratory Robot to perform routine residue analysis. Within the group was Work Permit Signatory and First Aider.

Promoted to Research Chemist in 1980 and Senior Research Chemist in 1984.

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1974 - 1977

Leeds Traffic Area (Civil Servant). Working in the typing pool as a junior shorthand typist. Responsible for typing accident reports, letters and minutes. Photocopying and telex operation. Preparation of files for archive and provision of a shorthand service to senior department heads.

1972 - 1974

Meanwood Park Hospital. Junior Shorthand typist. Provision of a shorthand and typing service to hospital management team. Responsible for production of menu and diet sheets for the catering division. Taking minutes of meetings and transcribing shorthand notes for circulation nationwide. Active participation in fund raising activities for the patients including playing the piano and organ at open days and fairs.

PHILLIPS

PE11

HAZLETON-UK

CURRICULUM VITAE

1. PERSONAL DETAILS

Name: MOORE, Jeff

Date of Birth: REDACTED

Job Title: Senior Scientist and Study Director, Department of Biopharmaceutical Analysis

Education:

1979-1982 Kingston Polytechnic
BSc Bioanalytical Science Option of Applied Science degree 2(i)

2. PRESENT EMPLOYMENT

Hazleton UK

REDACTED Senior Scientist and Study Director, Department of BioPharmaceutical Analysis (formerly Bioanalytical).
Responsibilities as for Study Director role (below) but since September 1991 responsible for Toxicology Support which include staff in Bioanalytical and in Toxicology Formulations; responsible to the Head of Department

REDACTED Study Director in Bioanalytical Department. Responsible for the supervision of projects within the department to ensure good

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2. PRESENT EMPLOYMENT - continued

REDACTED

scientific and commercial management. This includes close liaison with sponsors as well as the Section Manager. Supervision and training of junior and graduate staff is a further responsibility.

3. PAST EMPLOYMENT

REDACTED

Beecham Pharmaceuticals Research Division, Analytical Chemist in Pharmacokinetics Unit. Responsible for method development and routine assay of biofluid samples generated by toxicology studies and clinical trials as well as the reporting and pharmacokinetic interpretation of results. Responsible for training of junior staff in the use of VG Multichrom data capture system

4. TRAINING COURSES

1983	Pharmacokinetics Training Course (Dr P E Coates DMPD Beecham Harlow)
1984	Laboratory Animal Handling (introductory course for potential Home Office licensees)
1984	Solid-Phase Extraction Techniques (Analytical Symposium)
1984	VG Multichrom Training Course
1985	Communication and Report Writing Workshop

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4. TRAINING COURSES - continued

1986	Statistics for Industry : 2 modules i) Basic statistical techniques ii) Statistics for Research and Development
1986	Pharmacokinetics and Drug Disposition
1987	Management and Supervision training course eight 1-day modules (Scott-Grant)
1987	Report Writers Course
1987	Solid-Phase Extraction Techniques
1987	Total Quality Phase I Course (HUK)
1988	Report Writers Refresher Course
1988	International Symposium on Biomedical Applications of liquid chromatography (Bradford, UK)
1989	Total Quality Refresher Course (HUK)
1989	Chemistry Training Modules for Study Supervisors
1990	2nd International Symposium on Pharmaceutical and Biomedical Analysis (York, UK)
1990	18th International Symposium on Chromatography (Amsterdam, Netherlands)
1990	World Class Quality Course (HUK)

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4. TRAINING COURSES - continued

1991	Management Skills Training Course, HUK (5-day)

5. PROFESSIONAL QUALIFICATIONS

1989	Chartered Chemist and Member of Royal Society of Chemistry

6. PUBLICATIONS

1990	"Automation of a HPLC assay for the determination of nicotine, cotinine and 3-hydroxycotinine in human urine" J Biomed and Pharm Anal, 8: 1051-1054 (1990)
1991	Moore, J; Robotham, L and Wagner, J; "Tabulation of Bioanalytical Data: An alternative to LIMS", Chemometrics and Intelligent Laboratory Systems: Laboratory Information Management, 13:163-172 (1991)

Signature: _____ Date: 6.2.92.

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PE11

HAZLETON UK

CURRICULUM VITAE

1. PERSONAL DETAILS

Name:	PHILLIPS, Keith
Date of Birth:	REDACTED
Job Title:	Manager, Analytical Chemistry

Education:

REDACTED	'O' Levels in English Language, English Literature, Welsh, French, Geology, Physics, Maths, Chemistry, Special Arithmetic
REDACTED	ONC Denbighshire Technical College Chemistry, Mathematics, General Studies.
REDACTED	HNC Denbighshire Technical College Chemistry, Special in organic Synthesis.
REDACTED	GRIC University of Salford, Manchester (Part 1)
REDACTED	Elected Licentiate of the Royal Society of Chemistry
REDACTED	GRSC (by counselled experience).
REDACTED	Elected Fellow of the Royal Society of Chemistry.

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2. PRESENT EMPLOYMENT

Hazleton UK

REDACTED — Head of Analytical Chemistry
Responsible for Residues Chemistry, Physicochemistry, Chemistry Services. The service offered encompasses GC, HPLC, FTIR, GCMS, LCMS for the Agrochemical Industry.

REDACTED — Head of Chemistry Services
Responsible for the Instrument Laboratory, Mass Spectrometry facility, and Formulation Analysis comprising of Tobacco, Toxicology Support and Pharmaceutical Analysis. Responsible for scheduling and revenues projection for all sections within Chemistry Operations and provision of PBUs for business development. Also interfacing between operations and BD for present and forecasted workloads.

REDACTED — Head of Chemistry Operations
Responsible for all aspects of the administration, organisation and management of Chemistry Operations ie Analytical and Metabolic. This covers the actual performance, both scientific and financial, of all the projects carried out for clients within chemistry and meeting the standards laid down by company policy. The scope includes all the resources, human and physical attached to this area, and demands motivation of all the people involved.

Also responsible for ensuring the smooth and efficient day-to-day running of the profit centre by active participation and control and by maintaining a close liaison with the

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Director of Chemistry and the Principal Scientist for Metabolism and Pharmacokinetics in regard to proposal activities and project status.

REDACTED

Head of Central Dispensary
Initially made responsible for setting up the facility and resources to run it. Responsible for the management of all test articles received at Hazleton, in accordance with Good Laboratory Practice (GLP) regulations. The formulation of all the materials using detailed Standard Operating Procedures, training staff, preparation and implementation of career development programmes, competence check lists etc. Also responsible for all financial aspects of the department.

REDACTED

Senior Residues Analyst
Responsible for the development/evaluation and subsequent application of many analytical procedures for measurement of crop protection chemical residues. Project Manager for a series of plant and soil metabolism studies using mainly radiochemical techniques.

3. PAST EMPLOYMENT

REDACTED

Tobacco Research Council Laboratories
Analyst/Synthetic Chemist
Primarily concerned with investigations into the possible effects of tobacco smoking on health.
Responsible for the synthesis of an unsaturated precursor subsequently reduced using tritium gas to give a high specific

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radioactivity material for inclusion in the tobacco. Also involved with the evaluation of 2, 4-D and 2, 4, 5-T as endogenous cigarette smoke markers.

REDACTED

Wellcome Foundation, Berkhamsted
Principal Senior Technician
Responsible for the day to day management of all senior and junior technicians and reported directly to the Head of the Chemotherapy Unit. Actively involved in the synthesis of cyclopropane carboxylic acid derivatives (chrysanthemates) subsequently used for synthesis of pyrethroids. Gained wide experience of Grignard reagents during this period of employment.

REDACTED

Monsanto Chemicals Limited, Ruabon
Trainee technician - Senior Technician
During the first 12 months became acquainted with the handling and use of organic chemicals and received training in basic laboratory techniques.
Final position was as a senior technician reporting directly to a senior chemist. Gained experience in the synthesis of a wide range of anti-ozonant compounds and their subsequent testing using a rheometer for the studies of rubber vulcanisation reactions.

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4. TRAINING COURSES

1971-1972	Interpretation of NMR, UV, IR spectra held at Berkhamsted.
1974	Attended a residential course on radioisotope techniques at the University of Loughborough.
1980	Attended a residential Management Training Course.
1981	Report writing course for chemists.
1982	Management Training Course (1 week residential).
1983/4	Management/Supervison training course.
1985	Management Course/Time Management.
1986	Report Editors course for Chemists/Scientists.
1987	Total Quality Course.
1988	GLP in the Chemical laboratory RSC - ICI Wilton
1989	Quality Refresher Course.
1989	LIMS for the Chemistry Laboratory RSC London.
1989	Residue levels on crops and their analysis (BCPC London)
1989	PC training (Word Perfect).

1990	COSHH in laboratories.
1990	Management Skills course.
1991	Management Skills course.

REDACTED

6. SCIENTIFIC PUBLICATION

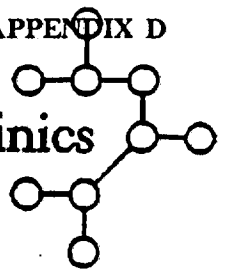
Synthesis of High Specific Activity Tritium Labelled Dotriacontane.
Journal of Labelled Compounds and Radiopharmaceuticals
Vol XIV, No 2 1978.

7. OTHER RELEVANT DETAILS

1978	Worked in a problem solving role at the Agrochemical Division of Bayer AG in Leverkusen West Germany in their Pesticide Residues Laboratory. This work in West Germany enabled Hazleton Laboratories to complete a project on behalf of the sponsor and submit a report to the PSPS.
1985	Co-author of Chemistry Division's Career/training programmed in chemistry.
1988	Development of HPLC/GC training course with Leeds Polytechnic. Subsequently modified and 25 chemists trained extensively in chromatography over 1-2 years.
1990	Developed brochure and overhead presentation on COSHH in laboratories for HUK. Presentation made to Health and Safety Executive.

1990	Member of the HUK COSHH Committee.
1991	Involved with RSC Chemistry at Work Exhibitions involving schools in Yorkshire.

GHBA/Hazleton Clinics
Leeds



INDEPENDENT REVIEW BOARD

CONSTITUTION

1. Name and Purpose

1.1 The Board shall be called the "Independent Review Board".

1.2 The responsibility of the IRB shall be:

- to protect the safety, rights and dignity of human subjects involved in clinical research studies
- to ensure the science warrants exposure of subjects to risk
- to ensure that applicable laws, regulations and standard operating procedures are followed.

2. Membership

2.1 The Board shall consist of twenty members.

2.2 The Board must include at least one woman and one man.

2.3 At least one member must be a registered medical practitioner.

2.4 At least one member must represent a non-scientific field.

2.5 A quorum consists of five members and must include a registered medical practitioner, a non-medical scientist and a non-scientist, both sexes should be represented.

2.6 Members shall serve for one year terms from 1 August. Consecutive terms of service are encouraged to provide continuity to the Board's activities.

2.7 It is the responsibility of the Managing Director of GHBA/Hazleton Clinic Leeds to nominate members of the IRB. The Chairman is appointed by the Managing Director of GHBA/Hazleton Clinic Leeds for a one year term which may be renewed at the MD's discretion.

3. Authority

3.1 The IRB has the sole power to approve, modify or disapprove all study protocols and informed consents for studies involving human subjects.

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3.2 Requirements for modification or disapproval of either a protocol or an informed consent may not be over-ruled by any number of GHBA/Hazleton's staff.

4. Meetings

Meetings are scheduled for the first and third Tuesday of every month at 6.30 p.m. Meetings are held at GHBA/Hazleton Clinic Leeds. If a meeting is not required, the Medical Director will inform the Chairman so Board members can be notified of the cancellation.

5. Voting

5.1 Voting will be oral upon a call of roll of the members present.

5.2 The decision of the IRB to approve or disapprove will be that expressed by a majority of the members present with all votes carrying equal weight. In the case of approval, at least one of the affirmative votes must be cast by a physician.

6. Reporting Results of Review

Results of an IRB review are submitted to the Principal Investigator on a pro forma, signed by the Chairman, or his alternate. This form will also include any requirements for amendment or modification to the protocol or informed consent.

7. Records

The IRB will be required to maintain:

7.1 Copies of all research proposal documentation submitted for review, together with completion notices detailing any adverse events.

7.2 Minutes of meetings to contain the names of members and others in attendance, decisions of the Board and the basis for disapproval of any proposal. Individual votes will be recorded.

7.3 A file containing name and qualifications of each member of the Board and their representative capacity.

7.4 A set of Standard Operating Procedures (both current and historical) which shall be circulated to each member and alternate at least once every twelve months.

8. Finance

8.1 Members shall receive travelling expenses for attendance at meetings.

8.2 GHBA/Hazleton will pay the sum of £15 per protocol reviewed either to each member or to a charity of their choice.

This Constitution is a resume of the Standard Operating Procedures by which the function of the IRB is regulated.

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